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Mineral Nutrition and Mycorrhizal Association of Bur Oak[1]

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The nutrient requirements of trees have been difficult to determine and interpret because of the complex structures involved in growth and the fact that roots occupy both the surface and subsoil. A further complication results from the union of soil fungi with absorbing roots to form specific mycorrhizal associations. These organs are favored in their development and occur most abundantly under undisturbed forest soil conditions. Thus the nutritional relations of tree species can be studied only when started from seed under specifically controlled conditions.

The investigation here presented was conducted to determine the effects of deficiencies in nitrogen, phosphorus and potassium on seedlings of bur oak, *Quercus macrocarpa* Michaux, in nutrient sand cultures free from the mycorrhizal association and the reaction of roots with mycorrhizal fungi in humus cultures. Bur oak was selected because of its wide adaptability to soil and climate. It occurs from Manitoba to Nova Scotia, southward to Georgia and Texas and westward to Wyoming and is the only pioneer species in the central prairie region.

MATERIALS AND METHODS

Seed of bur oak was collected in September from an isolated pure stand grove one and three fourths miles southwest of LeRoy, Indiana. After removing the cups, this was stored outdoors in wood boxes between layers of burlap with about three inches of moist sand below and above until January 15. These were brought into the greenhouse and thawed. For germination, single rows of 25 seeds each were arranged midway and wrapped into 6 inch burlap dolls then tiered upright in earthenware jars which were held with one-half inch of water in the bottom. These dolls, when wrapped and tied tightly, served as wicks to maintain uniform moisture on the surface of the seed coats. Enough seeds had germinated by February 15 to set up the sand and humus cultures.

Crystal grade silica quartz sand was washed and placed in 6 liter

[1]Investigation supported by American Potash Institute, Inc. and U. S. Department of Agriculture, Bureau of Plant Industry, Division of Forest Pathology.

earthenware crocks. Uniformly developed seedlings with tap roots about 3 inches in length were selected. The cotyledons were removed by clipping just before planting in the sand and humus cultures. Two seedlings were transferred to each culture so that the hypocotyl was exposed above the surface of the levelled sand. A modified Hartwell and Pember (3) solution was used for the nutrient cultures and contained the following salts in the basal solution: magnesium sulphate ($MgSO_4$) 0.0016 mol., ammonium nitrate (NH_4NO_3) 0.002 mol., potassium chloride (KC1) 0.0016 mol., calcium phosphate ($CaH_4(PO_4)_2$) 0.0004 mol., calcium nitrate ($Ca(NO_3)_2$) 0.003 mol., zinc sulphate ($ZnSO_4$) 0.000005 mol., manganese sulphate ($MnSO_4$) 0.00001 mol., and ferric citrate ($Fe_2(C_6H_5O_7)_2.6H_2O$) 0.00002 mol. For the deficiency cultures the following concentrations were used: low nitrogen, calcium nitrate and ammonium nitrate one-tenth or 0.0002 and 0.0003 mol. respectively, low phosphorus, calcium phosphate one-tenth or 0.00004 mol. and low potassium, potassium chloride one-twentieth or 0,00008 mol. Ten cultures of the basal and of each deficiency level were used in this series.

Humus cultures were made with leaf mold collected under the same isolated pure stand of bur oak from which the seed was obtained. The dry undecomposed leaves of the first layer were raked away and about three-fourths inch of the second or decomposing layer stripped away and packed moist for transporting in boxes. This was collected at the same time as the seed and stored in a damp, unheated basement until the seedlings were ready. After pulverizing and mixing, thirty clay 8 inch pots were filled and packed then planted with two seedlings each by the same method as the sand cultures. Half of these were moistened with the basal nutrient solution and half with tap water. All readings were made and roots placed in fixative (formalin-acetic-alcohol, Doak (2), p. 429) on May 15 and sectioned later. Several subsequent collections of bur oak mycorrhiza from locations in Pennsylvania, Indiana and Ohio were fixed and stained by other methods, Cohen and Doak (1), and compared with those from greenhouse humus cultures.

GROWTH RESPONSE AND DEFICIENCY SYMPTOMS

Seedling growth response differences were not considered significant due to the natural variation in the number and arrangement of the seedling leaves. The first group of leaves, which comprised the entire development for the duration of this experiment, consisted of a single whorl of 3 to 8 leaves or an occasional double whorl with 3 to 5 leaves in each. Diameter and length of the stems was extremely variable and showed no relationship to the nutrient deficiencies. Leaf color and size, however, showed striking and consistent differences at the nutrient levels maintained in sand cultures.

All seedling leaves in the low nitrogen cultures showed a general yellowish green color after 30 days while those in the basal cultures were light green or of much less intensity than normal for leaves in full sunlight. At this time most leaves in all cultures had attained about two-thirds the average size expected for bur oak. At the end of three months the low nitrogen leaves had not attained full size and

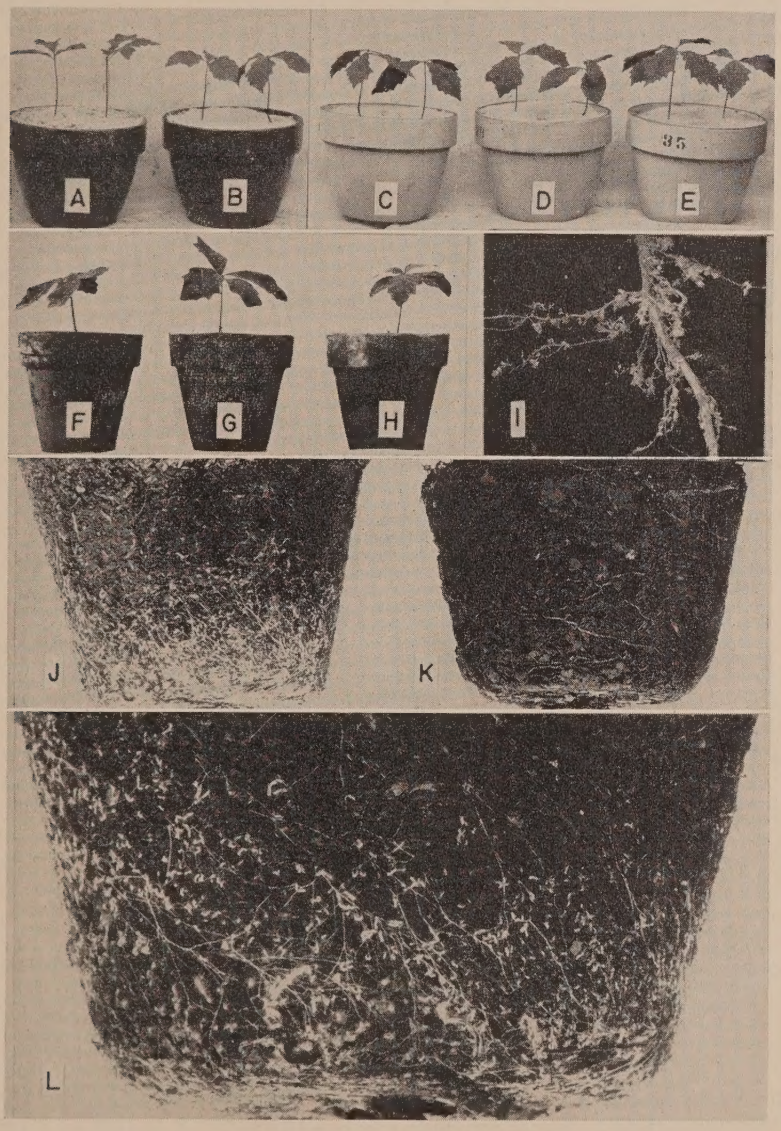

FIG. 1.—Quartz sand cultures of bur oak. A—Low nitrogen. B—Basal. C—Low phosphorus. D—Low potassium. E—Basal. Humus (leafmold) cultures. F and G—Humus alone. H—Humus with basal nutrient solution added. I—Tap root and mycorrhizal branches from culture F. J—Humus mass and roots removed from culture F, showing abundant mycorrhiza formation. K—Humus mass and roots removed from culture H, showing few and scattered mycorrhiza. L—Enlarged view of the humus mass and mycorrhiza from culture G, showing rhizomorphic strands passing between and attached to mycorrhiza and interspersed among the humus layers.

chlorosis was more pronounced (Figure 2, A and D). Intervenal and venal tissues were involved equally in this chlorosis.

Low phosphorus cultures at 30 days showed a leaf coloration indistinguishable from the basal. The youngest leaves, or those highest on the whorls, seemed slower in developing (in full sunlight in nature, the intensity of green color in early season as the leaves are growing increases proportionate to age and size of leaves). At the end of three months leaf size was extremely variable; some remained small while others attained size equal to the basal. Top leaves of the whorls remained the smallest and showed a reddish green color which was more intense along the midrib and primary veins (Figure 2, B and E). A few of the larger leaves showed this reddening to lesser degree.

In low potassium cultures, the size of leaves at the 30-day period was not perceptibly different from the basal. Sometime following this, a definite growth cessation occurred and at the end of three months the deviation from basal was almost equal to that of the low nitrogen cultures. A sharp intervenal chlorosis was more pronounced in the larger (usually lower) leaves of the whorls. The veins retained some green color and chlorosis at the points most remote from them was most extreme (Figure 2, C). This chlorosis consisted in a complete loss of color in contrast to the yellowish green developed in the low nitrogen cultures.

HUMUS CULTURES AND MYCORRHIZA

At the end of three months, the leaves of the group of humus (leafmold) cultures receiving basal nutrient were not distinguishable in color from those in humus alone. Upon removal of the root systems, which had bound the leafmold together, the cultures receiving basal solution showed only occasional and scattered mycorrhiza while most of the absorbing roots were mycorrhizal in the humus culture without mineral nutrient (Figure 1, J and K). After shaking the leafmold from roots and mycorrhiza, the total root systems including the mycorrhizal absorbing roots from the humus cultures without addition of the basal nutrient were found more extensive than the non-mycorrhizal root systems from cultures with basal nutrient.

Under the environment of the humus cultures here used, ectotrophic, monopodially branched, grayish mycorrhizae were developed in abundance only in those cultures lacking the mineral nutrient at basal concentration. Only the lateral or absorbing roots were involved; the extending or mother roots remaining uninfected. Mantles or enclosing fungus sheaths were usually slightly more than one half the thickness of the radial length of the epidermal cells of the host. This elongation of the epidermal cells did not occur in non-mycorrhizal roots in cultures of humus receiving the complete mineral nutrient. Intercellular hyphae penetrated in some instances to the first cortical layer (Figure 3, D-F).

In addition to the usual monopodial mycorrhiza, a compound form was collected from the bur oak stand from which seeds and leafmold were obtained. This was composed of a proliferation of absorbing roots near the tip of the extending roots. These entire clusters were surrounded by common mantles and appeared nodule-like with several

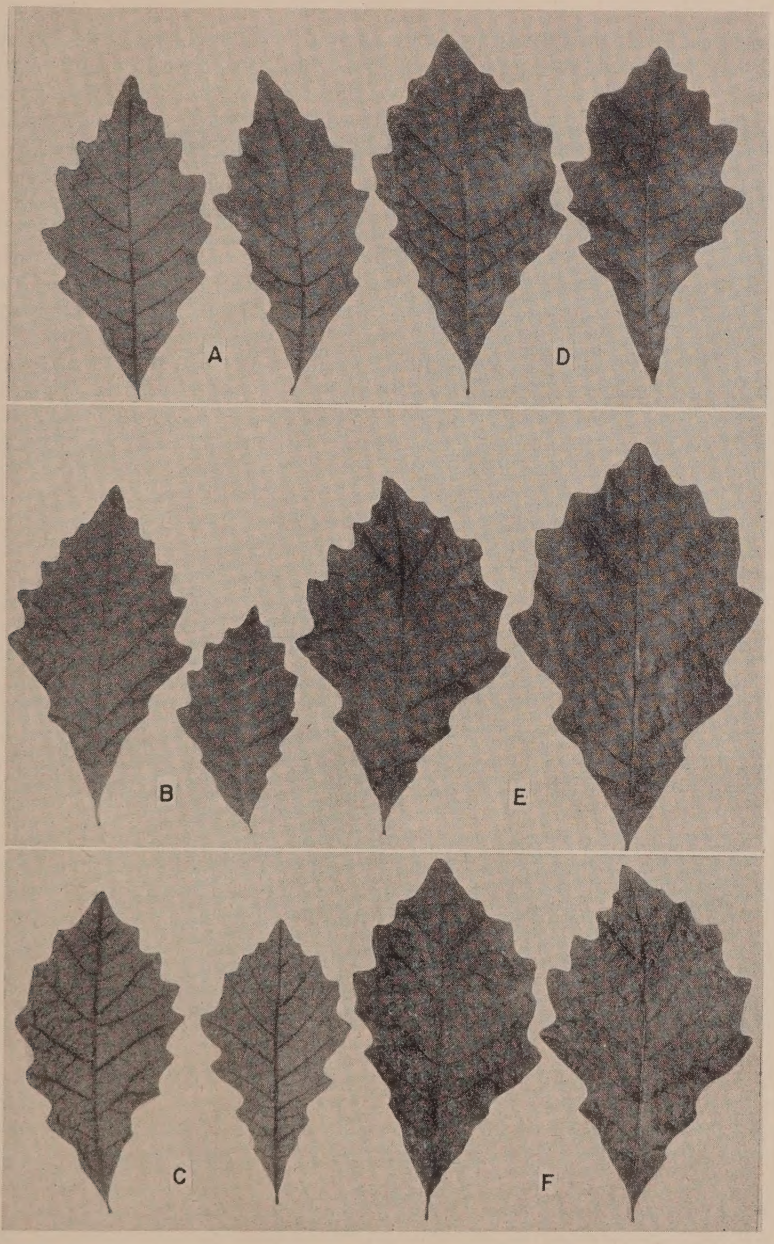

FIG. 2.—Leaves of bur oak from quartz sand cultures, in pairs receiving the same nutrient. A.—Low nitrogen, showing uniform general chlorosis of entire leaves. B—Low phosphorus, showing lack of chlorosis and extremes in size range (smaller leaves were reddish green in color). C—Low potassium, showing intervenal chlorosis D, E and F—Basal, showing fairly uniform distribution and intensity of color.

rhizomorphic strands passing from each to the mycelial mats in the adjacent humus layers (Figure 3, C). Mantle structure and intercellular penetration was similar to the monopodial form excepting that many of the individual root tips were so closely compacted that only thin mantle sheaths could be developed in the interspaces.

DISCUSSION

Any interpretation of the mineral deficiency symptoms found in these experiments must be made strictly on the basis of combinations of environmental factors lacking the mycorrhizal association. Open sand cultures here used had equal exposure to infection by air-borne spores of fungi, but the mineral nutrient alone was unfavorable for their development. All of the fungi thus far proven mycorrhizal are hymenomycetes and gasteromycetes requiring a medium of high organic content for development of the mycelium. The fact that bur oak seedling leaves in humus cultures without addition of mineral nutrient and with a well developed mycorrhizal system showed none of the deficiency symptoms found in sand cultures indicated that the three elements here considered were released from the humus by action of soil organisms, probably including the active mycorrhizal fungi, and absorbed either directly through the fungus mantle or the entire mycorrhizal system including the connecting rhizomorphs and mycelium. In sterile cultures of pine seedlings, Melin (8) demonstrated that organic nitrogen compounds not ordinarily available were changed by the action of mycorrhizal fungi and utilized by the host. By microchemical methods Masui (6, p. 227) found a concentration of potassium in the mycorrhizal mantles but less in the cortical cells of the mycorrhiza than in the cortex of non-mycorrhizal roots. Evidence of nutrient exchange and accumulation has not been correlated with growth reaction and deficiency symptoms.

Elongation of the epidermal cells seems characteristic of the bur oak mycorrhiza regardless of the causal fungi. Both the compound and simple monopodial types showed this reaction. Masui (5) described this on *Quercus paucidentata* Fr. and also illustrated an intracellular development, Figure 33, p. 180, which is exceptional among the oaks. The same radial elongation of the epidermal cells was reported for *Betula alba* by Paulson (9, p. 216) and for *Cupressus fastigiata* by Janse (4, p. 17, plate 15, figure 14). The stimulus for the development of the complex fungus mantle appears to be localized in the soil near the host roots. An accumulation of organic phosphorus compounds or phosphatides identified by Melin (7) might account for the intense localized development of mycelium. The nature of nutrient exchange through the mantle might explain many growth responses.

SUMMARY

Nitrogen deficiency in bur oak grown in sand cultures lacking mycorrhizal association was characterized by a chlorosis or yellowing accompanied by slight growth reduction. Phosphorus deficiency resulted in growth reduction and a reddish deep green coloration more prominently near veins of all leaves. With potassium deficiency intervenal chlorosis became locally complete near the outer edge of

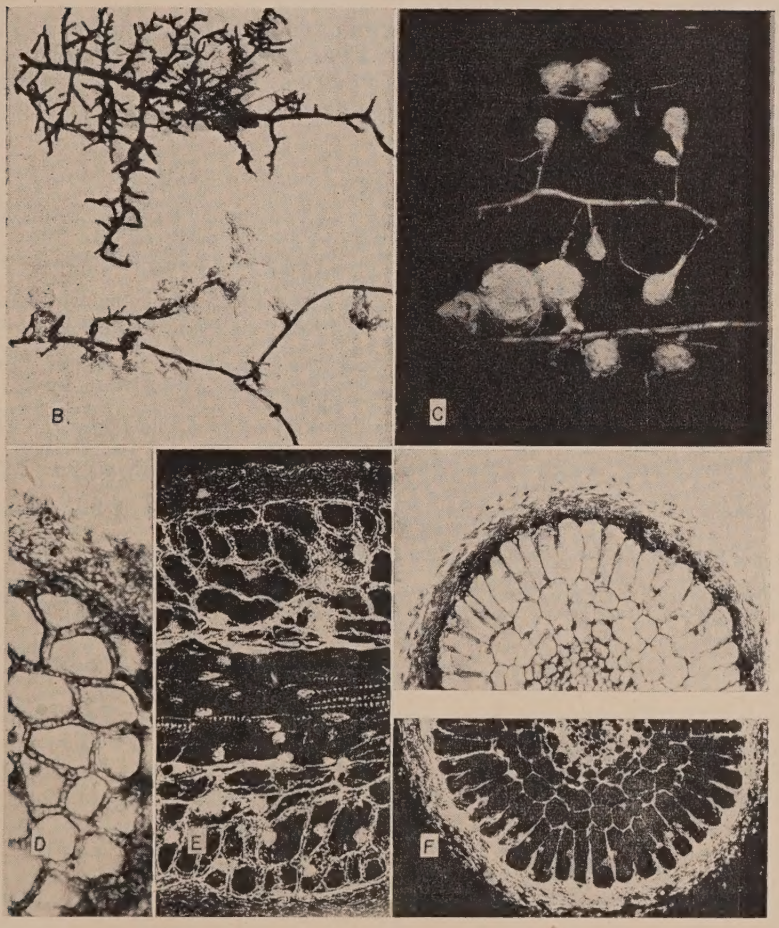

FIG. 3.—A—Roots from humus culture with basal nutrient added, showing abundant monopodial branching of absorbing roots and lack of mycorrhiza formation. B—Roots from humus culture without nutrient addition, showing white mycorrhizal mantles. C—Compound mycorrhizae composed of numerous tips surrounded by a common mantle. D—Longitudinal section through epidermis, showing intercellular hyphae, x 190. E—Longitudinal median section on dark field, showing intercellular hyphal development, x 130. F—Transverse sections, showing radial elongation of epidermal cells and hyphal penetration to first cortical layer, x 130.

leaves. Occasional intervenal necrosis was always preceded by yellowing, and accompanied slight reduction in leaf size. Stem length and diameter was variable and showed no relation to nutrient differences here used.

Mycorrhiza development was inhibited in humus cultures by addition of a complete mineral nutrient but growth and leaf color differences were insignificant. Mycorrhizal structures in the humus cultures lacking mineral addition were compared with those in nature morphologically.

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The Use of Glass Frit for the Hydroponic Culture of Tomatoes[1]

F. L. Wynd[2] and C. E. Wildon[3]

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I. INTRODUCTION

The availabilities of iron and manganese in very insoluble glass frits to various species of plants growing in hydroponic cultures have been reported by Wynd (1950, 1951, 1953a, b) and by Wynd and Stromme (1953). The effectiveness of these materials as carriers of trace elements when added to soil has been described by Wynd and Bowden (1951 a, b) and by Wynd and Stromme (1951).

The purposes of the present investigation were twofold. One was to extend the previous studies to include the response of tomatoes when their only source of iron and manganese was the frit used as the supporting medium in hydroponic cultures. The other was to determine if the frit had retained its ability to release iron and manganese to plants, even though it had produced a crop of roses, gardenias, asters and soybeans. The frit used in the present investigation had been leached with nutrient solutions maintained at various pH values almost continuously for three years.

II. METHODS AND MATERIALS

The hydroponic culture equipment was similar to that described by Wynd (1951). It was the same equipment, including the identical

[1]The expenses incurred by this investigation were borne by the Ferro Corporation, Cleveland, Ohio. The glass frit was compounded by Charles A. Vana, Research Chemical Engineer, Ferro Corporation.

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frit, that was used for the study of roses by Wynd (1953 a). The nutrient solution lacked iron and manganese, but otherwise was identical to that used in the investigations cited above. The composition of the solution was as follows:

SALT	GMS. PER LITER
$MgSO_4.7H_2O$	1.85
$Ca(NO_3)_2.4H_2O$	0.61
KH_2PO_4	1.23

Trace nutrients were added as indicated below:

FORM ADDED	NUTRIENT	PARTS PER MILLION
HBO_3	Boron	0.50
MO_3	Molybdenum	0.05
$CuSO_4.5H_2O$	Copper	0.02
$ZnSO_4.7H_2O$	Zinc	0.05

Well washed quartz gravel of the same particle size as the frit was the supporting medium for the *absolute control* cultures. The nutrient solution for these cultures contained 4 parts per million of iron and 0.5 part per million of manganese.

As in the previously reported experiments, each carboy of nutrient solution flooded simultaneously two culture pots; one contained frit and the other contained quartz. These quartz cultures served as the *frit controls*. They permitted comparison of the amounts of iron and manganese released into the solution by the solubility of the frit with the amounts released to the roots by contact with them.

The frit used in the present investigation had supported a crop of roses for many months, a crop of gardenias for several months, a crop of soybeans, and finally a crop of Asters. Nutrient solutions had been pumped through the frit every 4 hours for almost 3 years. The frit was prepared by Charles A Vana, Research Chemical Engineer for the Ferro Corporation of Cleveland, Ohio. It is designated in the records of the Ferro Corporation as A-6300-B. It contained 7.5 percent iron calculated as Fe_2O_3 and 3.0 percent manganese calculated as MnO_2.

The tomato seeds were germinated in flats in the greenhouse. They were planted March 12 and carefully transplanted into the experimental culture pots on March 30. At the time of transplanting, the plants were about 2 inches tall. The pH values of the solutions

were adjusted to the predetermined values of 4.0, 5.5 and 7.0 on April 3 and were re-adjusted to these values once each week throughout the growing period.

Two separate series of cultures were arranged at the same time. Each series was complete, but were at opposite sides of the greenhouse room. The data presented in the tables show certain differences between these groups of cultures. Three carboys of nutrient solution in each series were adjusted to each of the pH values, 4.0, 5.5, and 7.0. The nutrient solutions were replaced by freshly prepared solutions on May 15.

The tomato plants were harvested June 28. The stems, leaves, and fruit were separated, dried at 100° C., pulverized, and stored in air-tight containers until the chemical analyses were carried out.

The iron content of the dried material was determined by the method described by Hummell and Willard (1938). The manganese content was determined as recommended by Willard and Greathouse (1917).

III. EXPERIMENTAL RESULTS

A. Visual Appearance of the Plants

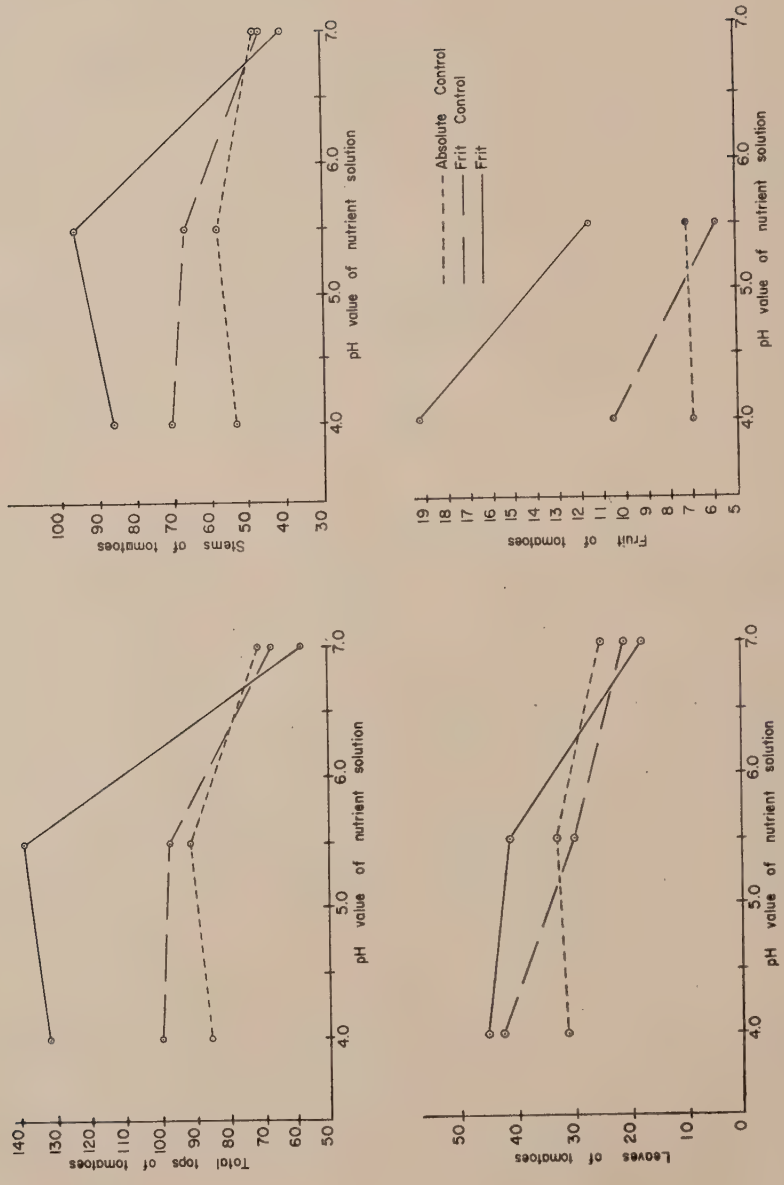
There were conspicuous differences in the visual appearances of the tomato plants in the various cultures within a few days after they had been transplanted. Those growing in the frit exhibited normal green color at all pH values of the nutrient solutions. The *frit control* cultures lost their original healthy appearance and became progressively more chlorotic as their growth continued. The *frit control* plants nourished by nutrient solution at pH = 7.0 were very inferior by the time they had been in the culture pots for 3 weeks.

Insofar as the eye could determine, the frit-borne plants growing in the nutrient solutions at pH values of 4.0 and 5.5 were equally thrifty, but those growing at pH = 7.0 obviously were retarded and, as was determined subsequently, failed to produce fruit. Visual observations suggested that the nutrient solutions at pH values of 4.0 and 5.5 were equally satisfactory and that pH = 7.0 was too high for satisfactory growth.

B. Dry Weights of the Plants

1. *Total tops.* The dry weights of the total tops, comprised of stems, leaves and fruit, are presented in Table 1 and Figure 1. At the pH values 4.0 and 5.5, conspicuously greater growth was attained by the plants growing in the frit. On the other hand, a favorable influence of the frit was not evident at pH = 7.0. The *frit control* plants were smaller than those growing in the frit, but were somewhat larger than the *absolute control* plants.

One might have expected that the *frit control* plants would have developed less well than the *absolute control* plants which received iron and manganese in their culture solutions. Since this was not true, it was evident that the *frit control* plants received some nutrients from


Absolute Control
Frit Control
Frit
Stems of tomatoes
Fruit of tomatoes
Total tops of tomatoes
Leaves of tomatoes
pH value of nutrient solution

their corresponding frit cultures by way of the leachate which flooded both simultaneously. Data presented below show that significant amounts of manganese had been leached from the frit and thereby made available to the *frit control* plants.

2. *Stems.* The data in Table 1 and Figure 2 show that the dry weights of the stems of the tomato plants followed the same trends, and exhibited the same relationships to the frit, as did the dry matter of the total tops.

3. *Leaves.* The dry weights of the leaves, just as the stems, also showed the same relative differences as did the dry weights of the total tops. The magnitudes of the differences, however, were smaller than in the instances of the total dry weights and the weights of the stems.

TABLE 1.—*Dry Weight of Tomato Plants, expressed in grams. Each Value Is the Average of 3 Plants, Each Grown in a Separate Culture.*

pH of nutrient solution		Total Tops			Stems			Leaves			Fruit		
		Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control
4.0	Exp. 1	88.4	135.6	92.8	53.9	87.9	64.6	34.5	47.7	28.2	5.98	24.18	11.43
	Exp. 2	82.5	129.9	108.2	53.5	86.6	77.1	29.0	43.3	31.1	8.03	14.43	8.68
	Ave.	85.5	132.8	100.5	53.7	87.3	70.9	31.8	45.5	29.7	7.01	19.31	10.06
5.5	Exp. 1	97.7	131.1	89.8	60.6	87.5	61.7	37.1	43.6	28.1	4.93	11.63	6 48
	Exp. 2	84.6	145.7	106.6	55.7	105.4	74.2	28.9	40.3	32.4	9.48	11.58	5.13
	Ave.	91.2	138.4	98.2	58.2	96.5	68.0	33.0	42.0	30.3	7.21	11.61	5.81
7.0	Exp. 1	66.3	50.0	69.6	44.3	37.4	48.2	22.0	12.6	21.4			
	Exp. 2	79.1	67.0	65.9	51.9	44.1	45.1	27.2	22.9	20.8			
	Ave.	72.7	58.5	67.8	48.1	40.8	46.7	25.6	17.8	21.1			

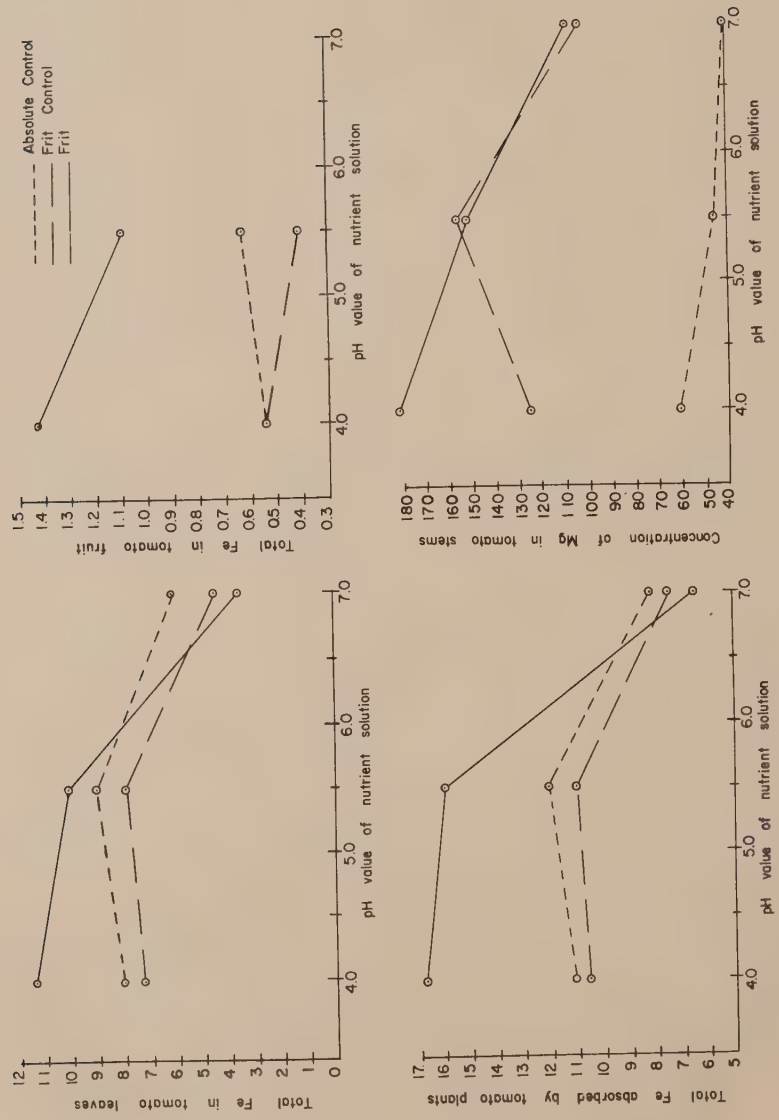
The fact that the stems were influenced favorably to a greater extent than were the leaves by the frit was not expected and seems, therefore, especially worthy of notice.

4. *Fruits.* No fruit was produced by the plants grown in nutrient solutions adjusted to pH = 7.0. At the lower pH values, however, the yields of fruit followed the same general trends as did the stems and leaves insofar as the effects of the frit were concerned. But, unlike the stems and leaves, the fruit developed significantly greater when the nutrient solution was maintained at pH = 4.0.

C. Iron Absorption

1. *Concentration of Iron in the Plants*

a. *Stems.* The data presented in Table 2 and Figure 5 show that the stems of the tomato plants grown in the frit contained a greater concentration of iron than did those of the *frit control* plants. The


Absolute Control
Frit Control
Frit
Total Fe in tomato fruit
pH value of nutrient solution
Concentration of Mg in tomato stems
pH value of nutrient solution
Total Fe in tomato leaves
pH value of nutrient solution
Total Fe absorbed by tomato plants
pH value of nutrient solution

magnitudes of the concentrations of iron in both the frit-borne and the *frit control* plants increased greatly as the pH values of the nutrient solutions increased. The dry weight of the stems also increased as the pH values of the solutions increased from 4.0 to 5.5, but decreased significantly at pH = 7.0. The fact that the development of the stems and the concentrations of iron they contained follow parallel trends when the pH values of the nutrients varied from 4.0 to 5.5, but sharply diverge when the pH was 7.0 indicated that the ability of the plants to accumulate iron was not the only factor limiting the amounts of stems produced.

The *absolute control* plants contained a slightly smaller concentration of iron as the pH values of the nutrient solutions increased.

TABLE 2.—*Concentrations of Iron in Tomato Plants, expressed as Parts per Million in the Dry Material. Each Value Is the Average of 3 Plants, Each Grown in a Separate Culture.*

pH of nutrient solution		Stems			Leaves			Fruit		
		Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control
4.0	Exp. 1	53.5	46.5	36.5	245.5	236.0	244.7	90.5	61.0	46.7
	Exp. 2	40.5	46.5	40.5	263.0	265.0	251.0	70.0	97.3	65.3
	Ave.	47.0	46.5	38.5	254.3	250.5	247.9	80.3	79.2	56.0
5.5	Exp. 1	43.5	56.7	38.7	251.0	241.0	230.7	108.0	89.0	64.3
	Exp. 2	38.5	42.7	43.0	300.5	241.0	286.0	77.0	97.7	76.3
	Ave.	43.5	49.7	40.9	275.8	241.0	258.4	92.5	93.4	70.3
7.0	Exp. 1	44.0	74.5	72.0	.....	164.3	182.7	.....		
	Exp. 2	41.0	64.3	55.0	322.5	230.7	252.3	.....		
	Ave.	42.5	69.4	63.5	322.5	197.5	217.5	.....		

b. *Leaves.* Data presented in Table 2 and Figure 6 show that the concentrations of iron in the leaves followed trends opposite to those exhibited by the stems. For example, the concentrations in the leaves of the frit-borne and their *frit control* plants lessened, rather than increased, as the pH values of the nutrient solutions approached neutrality. Also the concentrations in the leaves of the *absolute control* plants tended to be *greater*, rather than less, as the pH values increased.

It would appear that decreasing acidity of the nutrient solution lessened in some manner the ability of the tomato plants to transport iron from the stems and into the leaves. One may only surmise the mechanism by which this effect of pH might have operated. For example, the ions absorbed from the less acid (or more alkaline) solutions might render the cell sap more alkaline. This alkalinity might influence unfavorably the translocation of iron from the stems. This explanation admittedly is far fetched and lacks supporting data, but, at all events, the data obtained in the present study not only showed that the greater concentrations of iron in the stems were associated

with smaller concentrations in the leaves, but also showed that this association was related to the pH value of the nutrient solution.

c. *Fruits.* The data presented in Table 2 and Figure 7 show that the concentrations of iron in the tomato fruit increased as the pH values of the nutrient solutions increased. The fruit produced by the frit-borne and the *absolute control* plants contained very nearly the same concentrations of iron. These concentrations were significantly greater than in the fruit of the *frit control* plants.

2. *Total Iron Absorbed by the Plants*

a. *Stems.* Perhaps the most convincing evidence of the ability of the frit to release iron was the total amounts of iron absorbed by the plants. If the availability of iron had been the only factor limiting growth, the amount of iron absorbed automatically would have cor-

TABLE 3.—*Total Iron absorbed by Tomato Plants, Expressed as Milligrams and Calculated as Averages of 3 Plants, Each Grown in a Separate Culture.*

pH of nutrient solutions	STEMS			LEAVES			FRUIT			GRAND TOTAL		
	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control
Exp. 1	2.88	4.09	2.36	8.47	11.26	6.90	0.54	1.47	0.53	11.89	16.82	9.79
4.0 Exp. 2	2.17	4.03	3.12	7.63	11.47	7.81	0.56	1.40	0.57	10.36	16.90	11.50
Ave.	2.53	4.06	2.74	8.05	11.37	7.36	0.55	1.43	0.55	11.13	16.86	10.65
Exp. 1	2.64	4.96	2.39	9.31	10.51	6.48	0.53	1.04	0.42	12.48	16.51	9.29
5.5 Exp. 2	2.14	4.50	3.19	8.68	9.71	9.27	0.73	1.13	0.39	11.55	15.34	12.85
Ave.	2.39	4.73	2.79	9.00	10.11	7.88	0.63	1.09	0.41	12.02	15.93	11.08
Exp. 1	....	2.79	3.47	3.45	2.07	3.91	....	....	....	....	4.86	7.38
7.0 Exp. 2	2.13	2.84	2.48	8.77	5.28	5.25	....	....	....	10.90	8.12	7.73
Ave.	2.13	2.82	2.98	6.11	3.66	4.58	....	....	....	8.24	6.48	7.56

related positively with growth. Examination of the data in Table 3 and Figure 8 shows such a correlation did exist in the instance of the stems of the tomato plants grown in the frit. On the other hand, no such definite correlation existed for the *frit control* and *absolute control* plants.

It is interesting to note that when the total amounts of iron in the stems of the plants were compared with their concentrations, a totally different relationship appeared. For example, when the pH value of the nutrient solution was maintained at 4.0 and 5.5, these values correlated positively, but at pH = 7.0 the relationship was negative. One may infer from this situation that the amounts of iron in the stems correlated positively with their concentrations *if the plants were healthy and thriving*, as was the case when the pH values of the nutrient solutions were 4.0 and 5.5. But when the plants were

sickly and not thriving, the iron accumulated, but no corresponding augmentation of growth occurred. This failure of the plants to grow at a normal rate, even though iron was absorbed, leads to its greater concentration in the stems. Obviously, at pH = 7.0 some factor other than the availability of iron was limiting its movement into the leaves and the growth of the plants.

b. *Leaves.* It has been pointed out above that the iron in the stems became progressively less transportable into the leaves as the acidity of the nutrient solution decreased. Comparison of figures 3, 6 and 9 shows that this progressive immobility of iron is especially evident because the dry weight of the leaves, as well as the concentration and total amount of iron in their tissues, decreased as the pH value of the nutrient solution was increased. A positive correlation also appeared, although less markedly, in the leaves of the *frit control* and *absolute control* plants.

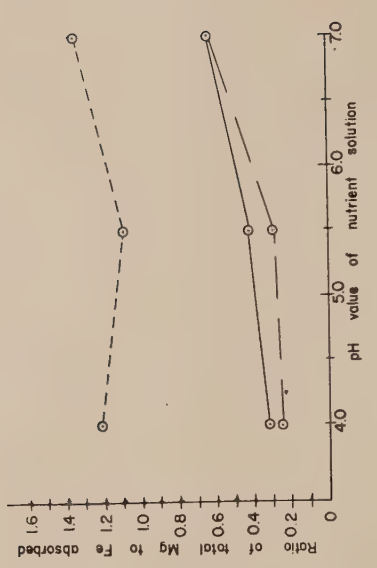
c. *Fruits.* Figures 4 and 10 show that the dry weights of the fruits and the total amount of iron they contained followed the same trend. For the frit-borne and *frit control* plants these values decreased as the pH values of the nutrient solution increased, but they increased slightly for the *absolute control* plants. The total iron in the fruits was inversely related to its concentration at pH = 5.5 because the yield of fruit decreased markedly at this pH value.

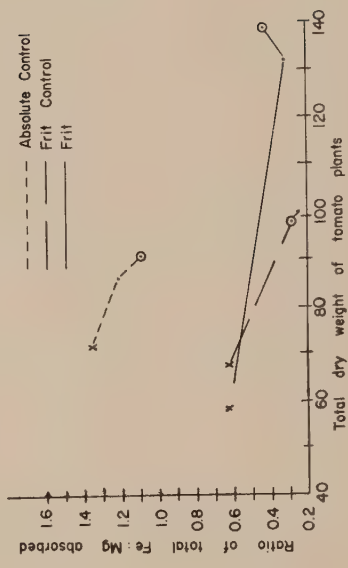
d. *Total plants.* Comparison of the sums of the total iron in the stems, leaves and fruits with the sums of the respective dry weights shows that both these values were high at the pH values 4.0 and 5.5, and conspicuously low at 7.0. Again it must be concluded, as in the instance of the concentration of iron, that the growth of healthy plants correlated positively with the amount of iron they absorbed, but that this was not true if some factor other than iron availability became a limiting factor.

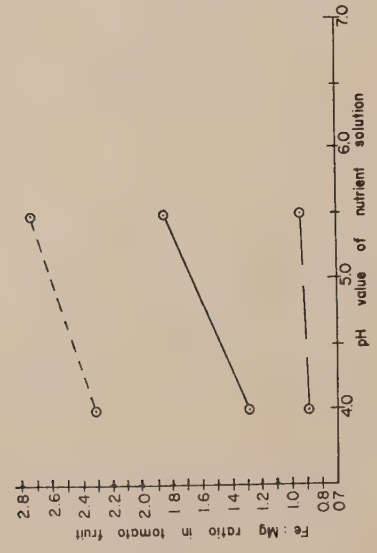
D. Manganese Absorption

1. *Concentration of Manganese in the Plants*

a. *Stems.* The concentrations of manganese in the stems of the tomato plants generally decreased as the acidity of the nutrient solutions decreased. As the data in table 4 and figure 12 indicate, this was especially evident in the frit-borne and *absolute control* plants. The concentrations in the frit-borne and the *frit control* plants were very much greater than in the stems of the absolute controls. These conspicuous differences show that considerable amounts of manganese were released to the frit cultures and also to their corresponding control cultures. The fact that the stems of the *frit control* plants contained very nearly the same concentration of manganese as did those of the frit-borne plants shows that the manganese in the frit must have been relatively soluble in the nutrient solutions. Previously reported analyses (Wynd, 1953 a) of nutrient solutions, after they had repeatedly flooded the culture pots filled with frit, showed that 17.0 parts per million of manganese accumulated in the solution at pH = 4.0, 11.3 at pH = 5.5 and 1.4 at pH = 7.0. It will be recalled that the concentrations of iron in the stems of the frit-borne and *frit control* plants


Ratio of total Mg to Fe absorbed
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
4.0
5.0
6.0
7.0
pH value of nutrient solution


Absolute Control
Frit Control
Frit
Ratio of total Fe : Mg absorbed
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
40
60
80
100
120
140
Total dry weight of tomato plants


Fe : Mg ratio in tomato fruit
0.7
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
4.0
5.0
6.0
7.0
pH value of nutrient solution

increased as the acidity of the nutrient solution decreased. This reciprocal relationship between iron and manganese has been reported by many authors.

b. *Leaves.* Table 4 and Figure 13 present data showing that the concentration of manganese in the leaves of the frit-borne and *frit control* plants rapidly decreased as the pH value of the nutrient solutions increased. This decrease was very much greater in the leaves than in the stems. It is especially noteworthy that the concentration of manganese in the *frit control* leaves was greater than in those of the frit-borne plants. These plants could not have had access to more manganese since their solutions received only the amounts

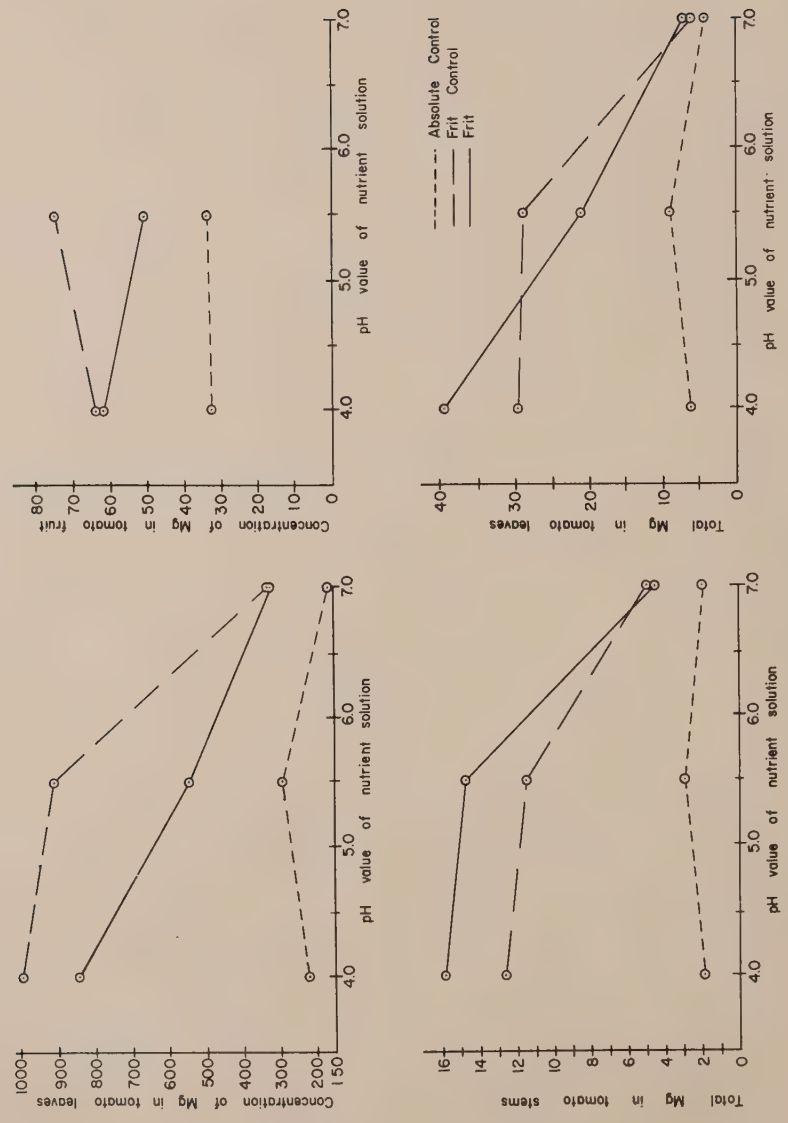
TABLE 4.—*Concentration of Manganese in Tomato Plants, Expressed as Parts per Million in the Dry Material. Each Value Is the Average of 3 Plants, Each Grown in a Separate Culture.*

pH of nutrient solution		STEMS			LEAVES			FRUIT		
		Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control
4.0	Exp. 1	68.5	172.5	140.5	217.5	604.5	920.5	39	48	55
	Exp. 2	54.0	190.7	210.0	220.5	1094.0	1070.0	26	76	72
	Ave.	61.3	181.6	125.3	219.0	849.3	995.3	33	62	64
5.5	Exp. 1	46.5	149.5	....	200.0	446.0	836.3	34	48	74
	Exp. 2	....	155.5	155.0	295.0	645.5	985.0	34	53	76
	Ave.	46.5	152.5	155.0	248.5	545.8	910.7	34	51	75
7.0	Exp. 1	44.5	119.5	104.0	160.0	358.3	315.7	..	..	..
	Exp. 2	36.5	99.7	104.0	173.0	294.3	345.0	..	..	..
	Ave.	40.5	109.6	104.0	166.5	326.3	330.4	..	.	..

leached from the frit cultures, but they certainly had access to *less* iron since it has been shown that this nutrient leaches from the frit only in very small amounts. The reciprocal relationship between iron and manganese mentioned above again was evidenced by the greater concentrations of manganese in the leaves when the plants had the least access to iron.

Comparison of figures 3 and 13 shows that the concentrations of manganese in the leaves of the frit-borne, *frit control* and also the *absolute control* plants correlated positively, within each series, with the dry weights of the leaves produced.

c. *Fruits.* The data presented in Table 4 and Figure 14 show that the concentration of manganese in the tomato fruits produced by the frit-borne plants was almost 100 percent greater than in the fruits produced by the *frit control* plants. The concentration decreased as the pH value of the nutrient solution increased; the opposite was true for the concentration of iron. Just as in the leaves, the *frit control* fruits contained a higher concentration of manganese than did those produced by the frit-borne plants, and probably is similarly explainable.


Concentration of Mg in tomato leaves
1000
900
800
700
600
500
400
300
200
150
4.0
5.0
6.0
7.0
pH value of nutrient solution
Concentration of Mg in tomato fruit
80
70
60
50
40
30
20
10
0
Total Mg in tomato stems
16
14
12
10
8
6
4
2
0
Total Mg in tomato leaves
40
30
20
10
0
Absolute Control
Frit Control
Frit

2. *Total Manganese Absorbed by the Plants*

a. *Stems.* The total amounts of manganese absorbed by the tomato stems appear in Table 5 and Figure 15. The amount decreased in the frit-borne and *frit control* plants as the pH value of the nutrient solutions increased. Although this decrease was small in the pH range from 4.0 to 5.5, it was great when the pH increased from 5.5 to 7.0.

The significant influence of pH values on the solubility of the manganese in the frit easily explains this decreased absorption. The relatively large amounts of manganese accumulated in the *frit control* stems, in contrast to the small amounts of iron absorbed, depended on the relatively greater solubility of the manganese.

TABLE 5.—*Total Manganese Absorbed by Tomato Plants, Expressed As Milligrams and Calculated As Averages of 3 Plants, Each Grown in a Separate Culture.*

pH of nutrient solution	STEMS			LEAVES			FRUIT			GRAND TOTAL		
	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control	Absolute Control	Frit	Frit Control
Exp. 1	2.36	15.16	9.08	7.50	28.83	25.96	0.23	1.16	0.63	10.09	45.15	35.67
4.0 Exp. 2	1.57	16.51	16.19	6.39	47.37	33.28	0.21	1.10	0.62	8.17	64.98	50.09
Ave.	1.97	15.84	12.64	6.95	38.10	29.62	0.22	1.13	0.63	9.14	55.07	42.89
Exp. 1	2.82	13.08	.....	7.42	19.45	23.50	0.17	0.56	0.48	10.41	33.09	.....
5.5 Exp. 2	....	16.39	11.50	8.53	26.01	31.91	0.32	0.61	0.39	.....	43.01	43.80
Ave.	2.82	14.76	11.50	7.98	22.73	27.71	0.26	0.59	0.44	11.06	38.08	39.65
Exp. 1	1.97	4.47	5.01	3.52	4.51	6.76	....	....	....	5.49	8.98	11.77
7.0 Exp. 2	....	4.40	4.69	4.71	6.74	7.18	....	....	....	.....	11.14	13.87
Ave.	1.97	4.44	4.85	4.15	5.63	6.97	....	....	....	6.12	10.07	11.82

The *absolute control* stems contained about the same amount of manganese at all pH values of the solutions. This is not too surprising when it is recalled that the amount available in these solutions was small andprobably was absorbed almost completely.

b. *Leaves.* Data presented in Table 5 and Figure 16 show that the total amount of manganese in the leaves of the frit-borne and *frit control* plants diminished rapidly as the pH values of the nutrient solutions increased, just as was the case with the stems. Also, as in the stems, about the same amount of manganese accumulated in the leaves of the *absolute control* leaves at all pH values of the nutrient solution. As would be expected, the amounts in the leaves were from 2 to 6 times the amounts in the stems. Comparison of figures 9 and 16 shows that the trends of the total amounts of manganese and iron in the leaves were similar as the pH values of the nutrient solutions increased.

c. *Fruits.* An interesting situation was found to exist in regard to the total amount of manganese in the tomato fruits. Data appearing in

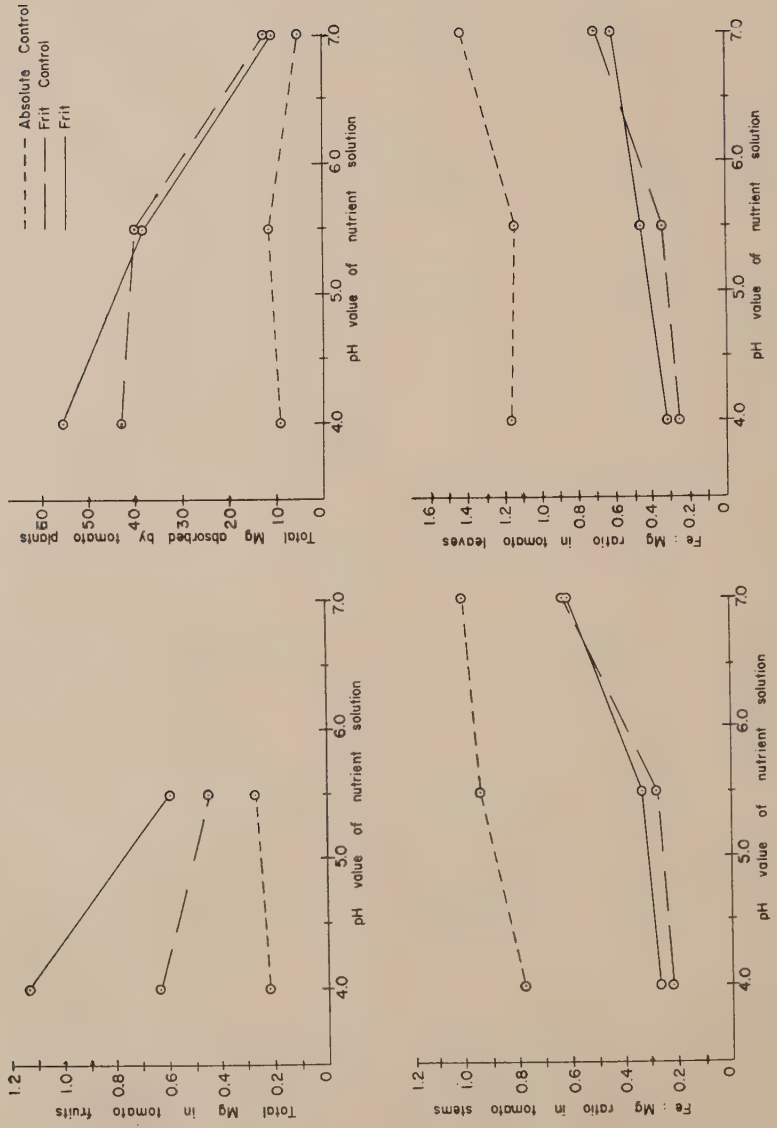

Absolute Control
Frit Control
Frit
Total Mg absorbed by tomato plants
Fe : Mg ratio in tomato leaves
Total Mg in tomato fruits
Fe : Mg ratio in tomato stems
pH value of nutrient solution

Table 5 and Figure 17 show that the amount lessened rapidly in the fruits produced by the frit-borne and *frit control* plants when the pH values of the nutrient solutions were increased from 4.0 to 5.5. Figure 10 shows that the same trends existed with respect to iron. Further, Figure 4 shows that they were very closely correlated with the yields of fruit.

d. *Total plants.* The total amounts of manganese accumulated in the stems, leaves and fruits appear in Table 5 and Figure 18. Since the amounts decreased in the stems, leaves and fruits of the frit-borne and *frit control* plants as the pH values of the nutrient solutions increased, the sum of these values also decreased. The total amounts of manganese absorbed by the *absolute control* plants are shown by Figure 18 to be about the same for all pH values of the solutions.

E. Ratios of Iron to Manganese in the Plants

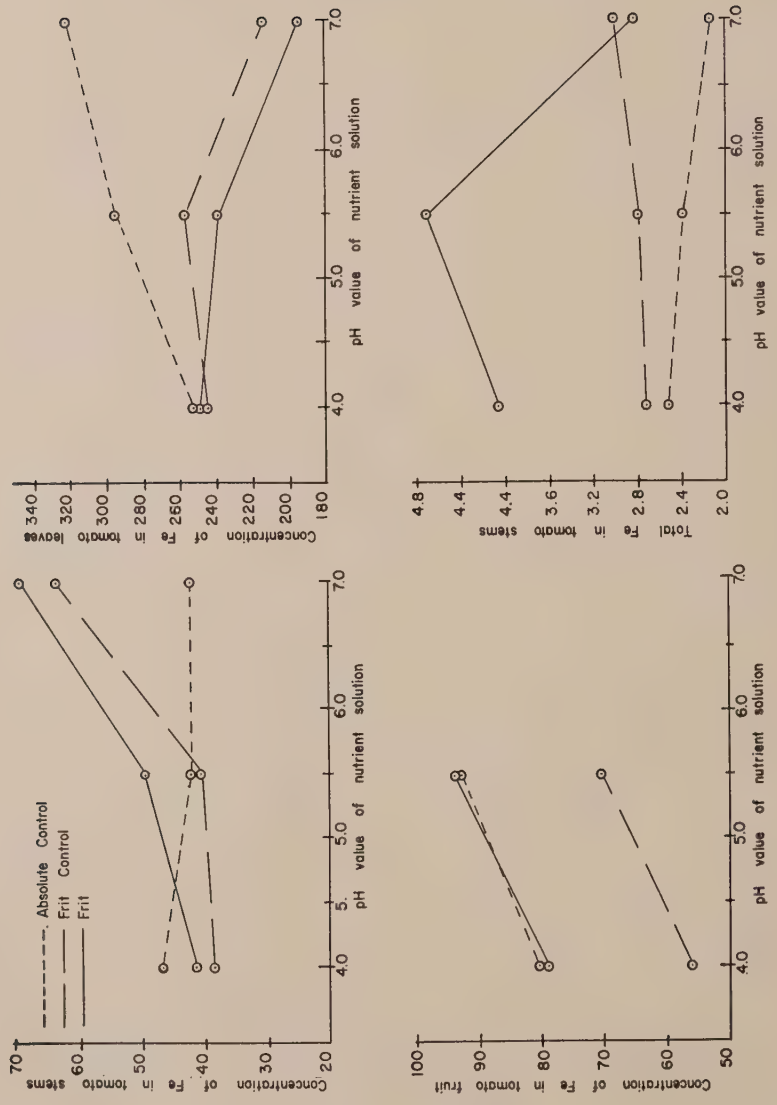
1. *Stems.* Among the most interesting results obtained by the present investigation were the small ratios of iron to manganese in all parts of the tomato plants. The data presented in Table 5 and Figure 19 show that the ratios varied from 0.77 to 1.05 in the stems of the *absolute control* plants. These values showed that the stems usually contained more manganese than iron, and that the amounts of manganese became relatively greater with respect to the iron as the pH value of the *absolute control* cultures were increased from 4.0 to 7.0. In other words, as the nutrient solutions approached neutrality, the accumulation of iron in the stems was inhibited to a greater extent than was the accumulation of manganese. This was true, in spite of the fact that the same amounts of both were present in the nutrient solutions.

The ratios of iron to manganese in the stems of the frit-borne plants were very much smaller than in those of the *absolute control* plants. The ratios exhibited by the *frit control* plants were still smaller. It is evident that the frit-borne plants had access to much more manganese than did the absolute controls. For example, there was about 3 times as much manganese as iron in their stems and the ratios of iron to manganese was from 2 to 5 times greater. These are remarkable and unexpected values.

The magnitude of the ratios increased as the pH values of the nutrient solutions decreased. This trend was to be expected in view of the earlier data (Wynd 1953 a) which showed that decreasing acidity of the nutrient solution lessened significantly the amount of manganese released by the frit, but did not correspondingly lessen the release of iron.

The ratios of iron to manganese in the stems of the *frit control* plants were even smaller than those of the frit-borne plants. This situation existed because considerable amounts of manganese reached these plants by way of the leachate from the frit cultures, but very little iron reached them from this source. Figure 19 shows that the trends of the ratios of iron to manganese in the stems of the frit-borne and the *frit control* plants were almost identical.

2. *Leaves.* The leaves contained from 3 to 4 times as much man-


Absolute Control
Frit Control
Frit
Concentration of Fe in tomato stems
Concentration of Fe in tomato leaves
Total Fe in tomato stems
Concentration of Fe in tomato fruit
pH value of nutrient solution

ganese as the stems, and from 4 to 8 times as much iron. The ratios of iron to manganese in the leaves are presented in table 6 and figure 20. The values for the *absolute control* leaves varied from 1.14 to 1.42. The value sharply increased when the pH value of the nutrient solution was maintained at 7.0.

The ratios of iron to manganese in the leaves of the frit-borne and the *frit control* plants were much smaller than in those of the *absolute control* plants which showed that they contained relatively very much more manganese. For the frit-borne plants, the value increased from 0.32 to 0.62 when the pH value of the nutrient solution was increased from 4.0 to 7.0. Evidently the leaves of these plants contained 2 or 3

TABLE 6.—*Ratios of Iron to Manganese in Tomato Plants, Calculated As Averages of 3 Plants, Each Grown in a Separate Culture.*

pH of nutrient solution	STEMS			LEAVES			FRUIT			GRAND TOTAL		
	Abso-lute Con-trol	Frit	Frit Con-trol	Abso-lute Con-trol	Abso-Frit	Frit Con-trol	lute Con-trol	Abso-Frit	Frit Con-trol	lute Con-trol	Abso-Frit	Frit Con-trol
Exp. 1	0.78	0.27	0.26	1.13	0.39	0.27	2.32	1.27	0.85	1.18	0.37	0.27
4.0 Exp. 2	0.75	0.24	0.19	1.19	0.24	0.23	2.69	1.28	0.91	1.29	0.26	0.23
Ave.	0.77	0.26	0.22	1.16	0.32	0.25	2.51	1.28	0.88	1.22	0.31	0.25
Exp. 1	0.94	0.38	....	1.26	0.54	0.28	3.18	1.85	0.87	1.20	0.50	....
5.5 Exp. 2	....	0.27	0.27	1.02	0.37	0.29	2.27	1.83	1.00	....	0.36	0.29
Ave.	0.94	0.33	0.27	1.14	0.46	0.29	2.73	1.84	0.93	1.09	0.42	0.28
Exp. 1	0.99	0.62	0.69	0.99	0.46	0.58	....	....	....	....	0.54	0.63
7.0 Exp. 2	1.12	0.64	0.53	1.86	0.78	0.82	....	....	....	....	0.73	0.56
Ave.	1.05	0.63	0.61	1.42	0.62	0.70	....	....	....	1.35	0.64	0.64

times as much manganese as iron! Figure 20 shows that the trend toward higher ratios was related to the decreasing acidity of the nutrient solution, and also that it was almost identical in the leaves of the frit-borne and *frit control* plants.

3. *Fruits.* Study of the data in Table 6 and Figure 21 discloses that only the fruits produced by the *absolute control* tomato plants exhibited ratios of iron to manganese of the order of magnitude ordinarily believed to be "normal". The value increased from 2.51 to 2.73 as the pH values of the nutrient solutions varied from 4.0 to 5.5. These fruits contained about 2.5 times as much iron as manganese. Since the same amounts of iron and of manganese were present in the *absolute control* solutions, it follows that the lessening of their acidities inhibited the absorption of manganese more than of iron.

The fruit produced by the frit-borne plants contained almost twice as much manganese per unit of iron than the *absolute control* fruits. The ratios of iron to manganese increased from 1.28 to 1.84 when the pH value of the nutrient solution varied from 4.0 to 5.5. The ratios

of iron to manganese in the fruits produced by the *frit control* fruits were even smaller than for those produced by the frit-borne plants. Their magnitudes were only 0.85 at pH = 4.0 and 0.93 at pH = 5.5. These values showed that there was considerably more manganese than iron in these fruits, due, of course, to the large amount of manganese and small amount of iron which reached the plants by way of the leachate from the frit cultures.

4. *Total plants.* The data in Table 6 and Figure 22 are based on the total amounts of iron and manganese absorbed by the stems, leaves and fruits of the tomato plants. Again, the greater ratios were exhibited by the *absolute control* plants, the values varying from 1.22 to 1.35. Greater amounts of manganese were available to the frit-borne plants, consequently their ratios varied from 0.31 to 0.64. Since the solubility of the manganese in the frit also supplied the *frit control* plants with large amounts of manganese, the ratios of iron to manganese in their fruits also were surprisingly low. The value varied from 0.25 to 0.64 and, in general, was lower than in the fruit of the frit-borne plants.

As figure 22 discloses, the decreasing acidity of the nutrient solutions increased the ratios of iron to manganese in all groups of the experimental plants.

IV. DISCUSSION

The data obtained during this investigation of the influence of the pH value of the nutrient solution on the availability of iron and manganese in glass frits to tomato plants disclosed several relationships of especial interest. One of these was that the concentration of iron in the plants correlated positively with growth, but *only* if other conditions were uniformly favorable. Plants which obviously were healthy and developing normally showed this positive correlation at pH values of 4.0 and 5.5. But when the pH value of the nutrient solution was maintained at 7.0, the plants did not thrive, even though they contained adequate concentrations of iron. Even though the pH value of 7.0 exerted a deleterious influence other than its influence on the availability of iron, one may not assume that this pH value was the specific cause of the poor growth. It is well known that tomatoes thrive in neutral and even calcareous soils exhibiting pH values of 8.0 or higher. The intricate chemical and physiological relationships to the concentration of hydrogen ions makes it all but impossible to separate cause from effect.

Throughout the growth period, the plants growing in the *frit control* cultures were distinctly chlorotic. It might be inferred that the chlorosis was caused by the lack of iron due to its great insolubility when incorporated into the frit and, consequently, its small concentration in the leachate from the frit culture. Chemical analyses of these chlorotic plants showed that they contained astonishingly large amounts of manganese. High concentrations of manganese, rather than the low concentrations of iron. undoubtedly caused the severe chlorosis that appeared.

The wide variations in the magnitude of the ratios of iron to manganese in the plants were among the outstanding results of the study.

Ordinarily, healthy plants contain more iron than manganese, but the largest and most vigorous plants obtained exhibited iron: manganese ratios as low as 0.3 or 0.4. That the plants were able to thrive, even though they contained several times as much manganese as iron, is surprising.

The data presented graphically in figure 23 show a strongly negative correlation between the total dry weights of the plants and the magnitudes of the ratios of iron to manganese in their tissues. But this was true *only* when the plants comprising a nutritional group are considered, that is to say, the frit-borne plants, the *frit control* plants, and the *absolute control* plants. The fact that the *absolute control* plants exhibited relatively high iron: manganese ratios approaching those generally expected in normal plants and yet failed to attain dry weights equal to the best frit-borne plants exhibiting the astonishingly low ratios of 0.3 or 0.4, showed that the magnitude of the ratio was not as important a factor as sometimes supposed.

Figure 23 shows graphically the large differences in the iron manganese ratios exhibited by the *absolute control* and *frit control* plants, yet the dry weights of these plants were about the same for any given pH value of the nutrient solution. The tolerance of high amounts of manganese by the tomato plants evidenced by their manganese content, as well as by the very low ratios of iron to manganese, was made vividly apparent by the analytic data obtained.

V. SUMMARY

1. Tomato plants were grown in hydroponic cultures with glass frit as the supporting medium. The frits contained 7.5 percent iron calculated as Fe_2O_3 and 3.0 percent manganese calculated as MnO_2. Two experiments were carried out to determine the effect of the pH value of the nutrient solution on the ability of the plants to obtain iron and manganese from the frit. Triplicate cultures, at each pH value, were used in each experiment.

2. Plants grown in the frit exhibited a normal green color at all pH values of the nutrient solution. Growth and development were normal at the pH values 4.0 and 5.5, but were greatly reduced at pH = 7.0. *Frit control* plants growing in quartz gravel flooded periodically with nutrient solution leached through the frit were distinctly chlorotic.

3. The stems of the plants growing in the frit contained more total iron and a greater concentration of iron than those growing in the *frit control* cultures. These amounts increased as the pH values of the nutrient solution increased. The opposite was true in respect to the leaves. Decreasing acidity of the solution caused iron to accumulate in the stems, and lessened its movement into the leaves.

4. The concentrations of manganese in the stems of the frit-borne and their corresponding *frit control* plants were about equal at specific pH values, but decreased rapidly as the pH values increased. There was a greater concentration of manganese in the leaves of the *frit control* plants than in their corresponding frit-borne plants. This situation probably resulted from the comparatively high solubility of the manganese and the low solubility of the iron. Increasing pH

values lessened the concentration of manganese in both the frit-borne and their *frit control* plants.

5. The total amount of manganese absorbed was greater in the stems of the frit-borne plants than in the stems of the *frit control* plants. The amounts rapidly lessened as the pH value of the nutrient solution increased. The total amounts of manganese in the leaves were about equal at specific pH values, but lessened as the values were increased.

6. The ratios of the amounts of iron to manganese were very much smaller in all parts of the plants grown in the *frit control* cultures than in those grown in the frit. This probably was due to the considerable amounts of manganese and the very small amounts of iron dissolved from the connected frit cultures. Healthy plants were obtained which exhibited iron:manganese ratios as low as 0.24 in the stem, 0.24 in the leaves, and 0.26 in the fruit.

7. In view of the current widely spread interest in the trace element content of human foods, it was of especial interest that the fruit of the frit-borne plants always contained significantly more iron and manganese than those grown in a complete nutrient solution containing 4.0 parts per million of iron and 0.5 part per million of manganese.

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Some New Indian Species of Synchytrium

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In a previous publication the author (1953) described three species of *Synchytrium* which were new to India. Since that time several additional species have been found which appear to be new and these were reported briefly before the Indiana Academy of Sciences by the author (1954). This paper gives the descriptions and diagnoses of these species. The present account is based upon study of living as well as fixed and stained materials. The specimens were collected around Banaras (India), from August, 1950 to January, 1953. Ten of these species have a long-cycle of development comprising prosori, sori, and resting spores, and the remaining three species are short-cycled producing only resting spores. Detailed studies on the germination of sporangia and resting spores and infection experiments are in progress at Purdue University.

Presence or absence of the sporangiosori and their mode of formation from the summer spores; presence of resting spores; morphological characters of fungus; obligate parasitism of *Synchytrium* species as restricted to hosts within related genera of the host family; characters of the sporangial and resting spore galls and over-all symptoms on the host; comparison of relevant details with similar species described from other countries and any other constant conspicuous features such as the dispersal of resting spores are the major criteria used by the author in considering these species as new. Intensive search for the host range in nature within the type locality was always kept in mind during the three years of collecting.

During the course of investigation of the microcyclic species which form only resting spores, a few isolated instances of empty galls which contained empty spore coats were observed. This indicates the possibility of a few spores germinating directly on the host, an unusual phenomenon in the subgenus *Pycnochytrium*. In such species fresh infection has been observed quite late in the season. It may be that the resting spores of the previous year which remained dormant in the soil may continue to germinate, few at a time, all through the season as is recorded in resting spore germination of *S. endobioticum* (Esmarch, 1927). However, the possibility of a few spores germinating during the same season on the living host cannot be ruled out and needs further investigation.

In 1945 Mhatre and Mundkur described 13 species of *Synchytrium* from specimens available in the Mycological Herbarium, Indian Agricultural Research Institute, New Delhi, including one on *Launea asplenifolia* and *Conyza* sp. as *S. vulgatum* Rytz. Inasmuch as it occurred on *Compositae* and appeared to form only resting spores resembling those of *S. vulgatum*, Mhatre and Mundkur's conclusion seemed justified. However, *S. vulgatum* Rytz is a short-cycled mem-

ber of the subgenus *Pycnochytrium* and parasitizes *Compositae* in Europe (Rytz 1907). Being a near relative of *S. aureum* Schroeter it produces only resting spores. The author collected what seemed to be the same fungus on *Launea asplenifolia*. Repeated examination of living material as well as prepared slides revealed the presence of prosori and sporangia (Figs. 2, 3, 5) in addition to the resting spores (Fig. 4) which were abundant later in the season. The author also examined the specimens deposited by various collectors since 1917 in the herbarium at New Delhi. While Butler's collections showed mostly resting spores, A. Khan's collections showed thin walled prosori, sporangia, and cupulate galls in abundance. However, A. Khan's note kept with the specimens mentioned only hypnospores. Comparisons of specimens of *S. aureum* and *S. vulgatum* obtained through the courtesy of Dr. Ernst Gäumann, Zürich, Switzerland and the author's discovery of sporangial stages in the life-cycle of this fungus lead to the conclusion that the fungus at hand is not *S. vulgatum* Rytz but a long-cycled species. Pending observation on the mode of germination of resting spores, this species is referred to the subgenus *Mesochytrium*, according to Karling's classification of the genus (1953). Accordingly, it is designated *S. launeae* sp. nov.

Infection of *Launea asplenifolia* occurs early in August. All aerial parts become disfigured with yellowish sporangial galls which are hemispherical and become cupulate on dehiscence of sori (Fig. 3). Reinfection and development of sori goes on during rainy months, resulting in extensive crustaceous thickening of the coalescent cupulate galls on all aerial parts of the host plant (Fig. 1). As the season advances, resting spores predominate. Very often a pink halo appears around hypnospore galls that are exposed to sun light. Development of sori and fresh infections were observed even in December when the dew drops were the only source of moisture.

Synchytrium launeae sp. nov.

Prosori sphaerici, singuli, dense lutei, leves, parietibus tenuibus praediti, germinantes, in vivis cellulis hospitis sporangiosoros gerentes. Sporangia 40–120 pro soro, ovata, sphaerica, 20 μ diam., citro-lutea. Hypnosporae sphaericae 50–138 μ (108 μ) diam., contentis citro-luteis globularibus. Exosporium 6 μ crassum. Gallae sporangiales, subsphaericae, 0.3–0.7 mm. diam., multicellulares, luteae, cupulatae, post dispersionem sporangiarum aggregatae, crustaceae. Gallae hypnosporarum subsphericae, 0.15–0.2 mm. diam., multicellulares, brunneae, rosalba corona vel defecta. Prosori et hypnosporae singuli vel multi pro galla, vel cellula hospita. Gallae in omnibus partibus foliorum incrassatae, luteae vel dense brunneae.

Prosori single, spherical, deep yellow, smooth, and thin walled. Sporangia 40–120 in a sorus, oval, spherical, orange-yellow and 20 μ in diam. Hypnospores single, 50–138 μ (108 μ) in diam. with orange-yellow globular contents. Exospore 6 μ thick. Sporangial galls subspherical 0.3–0.7 mm. in diam., composite, multicellular, yellow, cupulate after dehiscence of the sori, crowded, crustaceous and later turn dark brown. Hypnospore galls subspherical, 0.15–0.2 mm. in diam., multicellular, brown and with or without a pink halo. Prosori

and hypnospores one or more in a gall but one in a host cell. Galls on all parts of the foliage cause slight thickening, disfiguration and yellow to brown coloration.

On *Launea asplenifolia* Hook. f. Banaras, India. September 30, 1951. Leg. B. T. Lingappa. January, 1918. Pusa (Bihar), E. J. Butler; November 19, 1927. Pusa (Bihar), Sahaya; October 1932, Pusa (Bihar) A. Khan; December 15, 1917. Pusa (Bihar) Mukherji. (Figs. 1, 2, 3, 4, 5).

The second species to be reported here occurred on *Phyllanthus simplex* and *P. urinaria*. The greenish galls are crowded near the stem apices and along the long rachis of *P. simplex* which becomes slightly thickened whereas on *P. urinaria* the over crowded galls cause defoliation and incite conspicuous hypertrophy of the affected parts (Fig. 6). The affected stem and branches of *P. urinaria* become 3–4 times thicker than the normal. The hypertrophied parts are yellowish and turn brown later. Though the symptoms are markedly different on the two hosts the similarity of the characters of the prosori and hypnospores of the parasite lead to the conclusion that the same fungus incites different symptoms on two different hosts. The average larger size of the hypnospores on *P. simplex* (Fig. 7) is considered to be due to sparse infection of the host and the consequent greater nourishment available for the growth of the fungus. The hypnospores from the crowded galls on the midrib of *P. simplex* were slightly smaller than those from the folicolous galls which were generally separate.

Synchytrium phyllanthi sp. nov.

Prosori sphaerici, pallidi, fulvi, leves, vesiculosi, parietibus tenuibus praediti germinantes sporangiosoros gerentes. Sporangia 40–70 pro soro, ovata vel sphaerica, 33 μ diam., et virido-lutea. Hypnosporae sphaericae, fusco-brunneae, 66–120 μ (102 μ) in diam. in *P. urinaria;* 120–160 μ (146 μ) diam. in *P. simplex;* contentis luteis globularibus. Exosporium 7 μ crassum, fuscum, contentis cellulae hospitae defunctis densum. Gallae sporangiales multicellulares, subsphaericae, 0.3–0.5 mm. diam. Gallae hypnosporarum subsphaericae, multicellulares, 0.15–0.2 mm. diam. Prosori vel hypnosporae singuli vel multi pro galla singuli pro cellula hospita. Gallae in omnibus partibus foliorum *P. urinariae* incrassatae.

Prosori spherical, solitary, thin walled and light brown. Sporangia 40–70 in number, oval or spherical, 33 μ in diam. and greenish yellow. Hypnospores spherical, dark brown, 120–160 μ (146 μ) in diam. in *P. simplex* and 66–120 μ (102 μ) in diam. in *P. urinaria;* with yellow globular contents. Exospore 7 μ thick, dark brown with a thick deposition of denatured host cell contents. Sporangial galls subspherical, 0.3–0.5 mm. diam., multicellular, glistening yellow, cupulate after dehiscence of the sori, composite and coalescent. Hypnospore galls subspherical, multicellular, single or composite and 0.16–0.2 mm. diam. Prosori and hypnospores develop singly in host cells but one or more in a gall.

On *Phyllanthus simplex* Retz. and *P. urinaria* Linn. September 14, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 6, 7, 8).

Synchytrium crustatum sp. nov.

Prosori sphaerici, lutei, leves, parietibus tenuibus praediti, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 30–100 pro soro, ovata, sphaerica vel pyriformia, 18–21 μ diam. et lucido-citrea. Hypnosporae sphaericae vel oblongae, fuscae, 66–115 μ (95 μ) diam., contentis luteis globularibus. Exosporium 8 μ crassum, contentis cellulae hospitae defunctis densum. Gallae sporangiales, hemisphaericae, multicellulares, 0.4–0.6 mm. diam., luteae. Gallae hypnosporarum hemisphaericae, separatae vel aggregatae, multicellulares, 0.2–0.4 mm. diam., brunnaea. Hypnosporae vel prosori singuli vel multi pro galla, vel cellula hospita. Gallae in omnibus partibus incrassatae.

Prosori spherical, yellow, thin walled and smooth. Sporangia 30–100 per sorus, bright orange, oval, spherical or pear shaped and measure 18–21 μ diam. Hypnospores spherical, oblong 60–115 μ (95 μ) diam., brown with yellow globular contents. Exospore 8 μ thick with a thick deposition of denatured host cell contents. Sporangial galls, multicellular, composite, hemispherical, 0.4–0.6 mm. diam., yellow, later cupulate after dehiscence of the sori, coalescent, crustaceous and dark brown. Hypnospore galls multicellular, subspherical, 0.2–0.4 mm. diam., brown, intermixed with the crustaceous sporangial galls or rarely scattered. Prosori and hypnospores one or more in a gall and one or more in a host cell.

Galls on all aerial parts causing hypertrophied patches on stem, petioles, inflorescence and fruits of *Indigofera enneaphylla* Linn. and *I. liniola* Retz, October 3, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 9, 10, 11).

The infected parts show crustaceous patches with considerable hypertrophy (Fig. 9). These patches consist of numerous erumpent sporangial galls which are yellow at first and later become brown. The hypnospores when developed in these crusts are over crowded and at maturity with the partial disintegration of the infected host cells and appear to lie in large numbers in a single cavity (Fig. 11). More than one hypnospore also may develop in a single host cell. Infection of *I. liniola* plants in the neighbborhood incites only very mild symptoms. Numerous other leguminous plants growing in the immediate

EXPLANATION OF PLATE I

PLATE 1.—FIGS. 1–5. *Synchytrium launeae*. 1. *Launea asplenifolia.*, symptoms on the foliage. x 1/3. 2. Sporangia from mature sorus. x 300. 3. Cupulate gall, sorus and developing thalli. x 60. 4. Resting spore gall containing a resting spore. x 70. 5. Mature sorus dissected from a fresh gall. X 60. FIGS. 6–8. *S. phyllanthi*. 6. *Phyllanthus urinaria* plant showing hypertrophied symptom. x 1/4. 7. T. S. of leaf of *P. simplex* showing gall containing a resting spore. x 39. 8. T. S. through the hypertrophied stem of *P. urinaria* showing galls, prosori, sori and resting spores. x 50. FIGS. 9–11. *S. crustatum*. 9. *Indigofera enneaphylla* plant showing crustaceous patches. x 1/4. 10. Cupulate gall. x 40. 11. T. S. through the crust showing the galls, sori, young thalli and resting spores. Note the cavity in which the resting spores lie together. x 60. FIGS. 12–15. *S. zorniae*. 12. *Zornia diphylla* plant showing early stages of infection. x 1/3. 13. Galls containing a prosorus and a sorus. x 60. 14. Resting spore in a unicellular gall. x 70. 15. Prosoral gall, note the denatured contents of the surrounding cells. x 60. FIGS. 16–17. *S. oroxyli*. Showing stages of sorus formation in the vesicle extruded from the prosorus. 16. The multinucleate stage. x 120. 17. Development of cleavage planes. x 120.


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vicinity remained uninfected though the conditions were equally favorable for infection.

Synchytrium zorniae sp. nov.

Prosori sphaerici, parietibus tenuibus praediti, viridi-lutei, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 60–90 pro soro sphaerica, 20 μ diam. lutea. Hypnosporae sphaericae, oblongae vel ovatae, 95–190 μ (160 μ) diam., fusco-brunneae, contentis luteis globularibus. Exosporium 5 μ crassum. Gallae sporangiales crateriformes, 0.2–0.4 mm. diam., multicellulares, aggregatae, luteae, corona rubicunda vel defecta, post dispersionem sporangiorum cupulatae, brunneae. Gallae hypnosporarum unicellulares 0.1–0.15 mm. diam., brunneae. Prosori vel hypnosporae singuli pro galla vel cellula hospita. Gallae in omnibus partibus, partes infectae haud vel leviter incrassatae.

Prosori spherical, thin walled, greenish yellow. Sporangia 60–90 in a sorus, yellow, spherical, 20 μ diam. Hypnospores spherical oval or oblong, 95–190 μ (160 μ) diam., dark brown with yellow globular contents. Exospore 5 μ thick. Sporangial galls crateriform, composite, multicellular, 0.2–0.4 mm. diam., yellow with or without a pink halo and cupulate after dehiscence of the sori, coalescent and brown. Hypnospore galls composite, mostly on the under surface of the leaves, subspherical, 0.1–0.15 mm. diam., unicellular or multicellular, less conspicuous than the sporangial galls. Prosori and resting spores one in a gall and one in a host cell.

On all aerial parts of *Zornia diphylla* Pers. September 10, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 12, 13, 14, 15). Light green galls on the leaves and the petioles of *Zornia diphylla* plants indicate the early stages of infection (Fig, 12). At maturity these galls show yellow spots in the center denoting the mature prosorus. The mucilagenous contents of the cells surrounding the infected cell sometimes become pink and provide an attractive halo. On the dispersal of sporangia the galls become cupulate and cause slight deformation of the leaves. Hypnospores incite moderate gall formation (Fig. 14).

EXPLANATION OF PLATE II

PLATE 2.—FIGS. 18–20. *S. oroxyli*. 18. *Oroxylon indicum* shoot covered with galls. x 1/3. 19. Gall containing a resting spore. x 50. 20. Cupulate gall and a sorus. x 55. FIGS. 21–24. *S. trichodesmatis*. 21. Intact sorus dissected from fresh gall. x 55. 22. Sporangial gall containing a sorus. x 66. 23. Gall containing a resting spore. x 57. 24. *Trichodesma indicum*, shoot showing the symptoms. x 1/3. FIGS. 25–28. *S. maculans*. 25. *Sida rhombifolia*, shoot covered with galls. x 1/3. 26. A sorus dissected from a fresh gall. x 48. 27. A cupulate gall. x 30. 28. Galls containing resting spores. x 55. FIGS. 29–32. *S. oldenlandiae*. 29. *Oldenlandia corymbosa* plant showing severe symptoms. x 1/3. 30. Mature sporangia. x 430. 31. Gall containing a resting spore. x 35. 32. Cupulate gall and sori. x 25. FIGS. 33–36. *S. biophyti*. 33. *Biophytum reinwardtii*, plant bearing galls. x 1/3. 34. Gall containing a resting spore. x 60. 35. A mature and an empty sporangium. x 480. 36. Gall containing a sorus. x 75.


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Synchytrium oroxyli sp. nov.

Prosori spherici, lutei, parietibus tenuibus praediti, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 3–100 pro soro, ovata vel spherica, lutea. Hypnosporae sphericae, 100–150 μ (130 μ) diam., brunneae, leves, contentis lucido-luteis. Exosporium 5 μ crassum. Gallae sporangiales subsphericae, multicellulares, 0.5 mm. diam., post dispersionem sporangiorum cupulatae, aggregatae, crustaceae, primo aspectu aeciis uredinarum similes. Gallae hypnosporarum papillatae, multicellulares, 0.3–3 mm. diam. Gallae simplices. Partes infectae incrassatae, nigrescentes, emortuae.

Prosori spherical, yellow, thin walled. Sporangia 3–100, spherical or oval and yellow. Majority of sporangia measure 30 μ diam. Hypnospores spherical, smooth, 100–150 μ (130 μ) diam., brown with bright yellow contents. Exospore 5 μ thick. Sporangial galls spherical, composite, 0.5 mm. diam., light yellow, multicellular, on dehiscence cupulate and crustaceous. Hypnospore galls papillate, simple, hard, brown separate or crowded, multicellular, 0.3–3 mm. diam. Affected parts become distorted, brown, hypertrophied and die out.

On tender aerial parts of *Oroxylon indicum* Linn. August 3, 1950. Banaras, India. Leg. B. T. Lingappa. (Figs. 16, 17, 18, 19, 20 and 47, 48, 49 and 50).

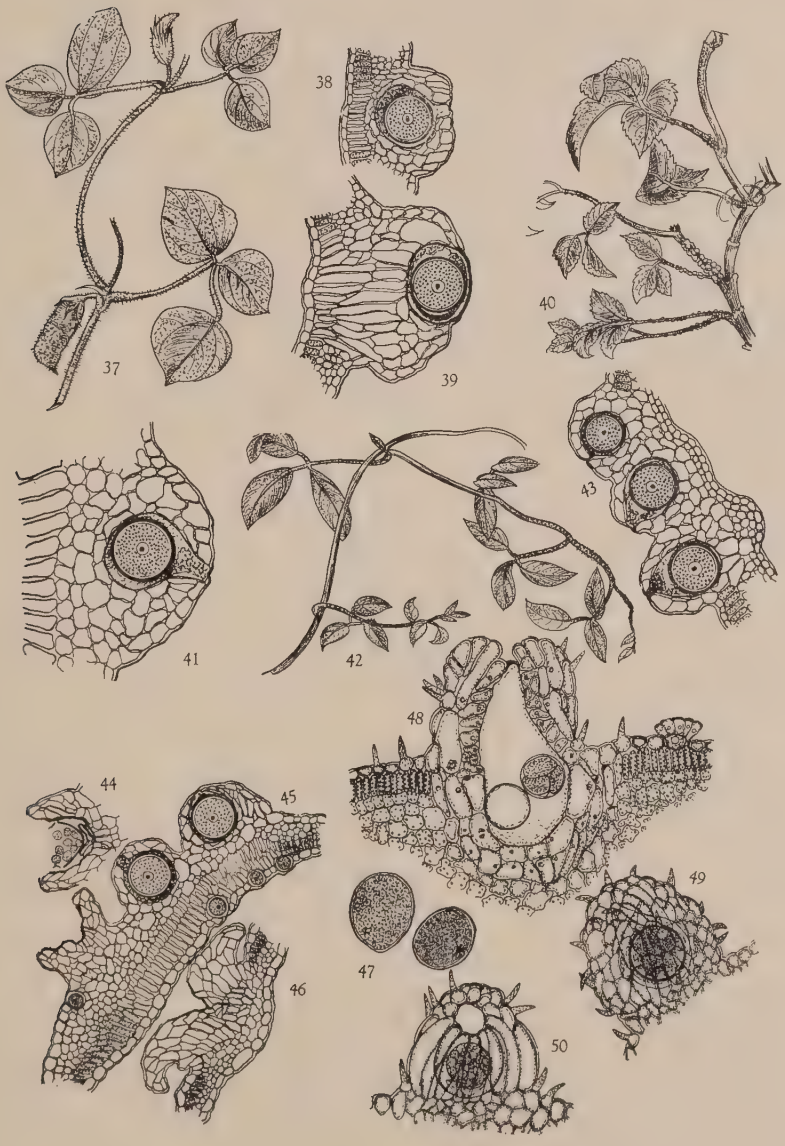
This species incites symptoms very much similar to the aecial stages of systemic plant rusts on all tender aerial parts (Fig. 18). The host plants are attacked when they are not more than 3 feet high. Prosori produce generally 60–100 sporangia but exceptional cases of only 3 sporangia were observed (Fig. 20, 48, 50). Hence the size of the sporangia vary greatly. The size and shape of sporangia fluctuate depending upon whether or not they are just released from the sori and this type of variation is common for all other species as well. In rare cases resting spore galls are as big as 3 mm. in diameter conspicuously papillate and hard (Fig. 49). Such galls were sparsely scattered and contained normal resting spores.

Synchytrium trichodesmatis sp. nov.

Prosori sphaerici, parietibus tenuibus praediti, leves, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 40–70 pro soro, pyriformia, 19 x 24 μ magnit., citrina. Hypnosporae sphaericae, 50–120 μ (90 μ) diam., leves, brunneae, contentis luteis globularibus. Exosporium 5 μ crassum. Gallae sporangiales subsphaericae, multi-

EXPLANATION OF PLATE III

PLATE 3.—FIGS. 37–39. *S. rhynchosiae.* 37. *Rhynchosia aurea*, a portion of vine covered with galls. x 1/6. 38. An immature gall containing a resting spore. x 48. 39. Mature gall, before the dispersal of the resting spore. x 46. FIGS. 40–41. *S. viticola.* 40. A tender shoot of *Vitis trifolia* bearing the galls. x 1/3. 41. Gall containing a resting spore. x 66. FIGS. 42–43. *S. thirumalachari.* 42. A portion of *Atylosia scarabeoides*, covered with galls. x 1/4. 43. Galls containing resting spores. x 44. FIGS. 44–46. *S. cassiae.* 44. Sporangial gall containing a portion of sporangiosorus. x 65. 45. Cupulate and resting spore galls. x 80. 46. Proliferated old cupulate gall. x 50. FIGS. 47–50. *S. oroxyli.* 47. Mature sporangia. x 330. 48. A fresh gall containing a sorus of 3 sporangia. x 48. 49. Full view of an isolated fresh gall containing a resting spore. x 70. 50. A full view of another type of fresh gall containing a sorus of numerous sporangia. x 60.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cellulares, 0.3–0.5 mm. diam., luteolae, cupulatae, post dispersionem sporangiorum aggregatae, ovatae, ellipticae et crustaceae. Gallae hypnosporarum minutae, 0.1–0.2 mm. diam., subsphaericae, brunneae, plures hypnosporae nullas gallas gerentes. Prosori et hypnosporae singuli vel multi pro galla, vel cellula hospita. Gallae in omnibus partibus densae et luteae.

Prosori spherical, thin walled and yellow. Sporangia 40–70 per sorus, pyriform, 19 x 24 μ, light orange. Hypnospores spherical, 50–120 μ (90 μ) diam., deep brown, smooth, with yellow globular contents. Exospore 5 μ thick. Sporangial galls, subspherical, 0.3–0.5 mm. diam., light yellow, multicellular, cupulate, oval or elongate after dehiscence and become crustaceous. Hypnospore galls, unicellular, undeveloped and inconspicuous or multicellular. Prosori and hypnospores one or more in a gall, one in a host cell.

On all aerial parts of *Trichodesma indicum* R. Br. August 10, 1950. Banaras, India. Leg. B. T. Lingappa. (Figs. 21, 22, 23, 34).

This species persists on its host till late in December, evidently capable of utilizing the dew drops for fresh infection. Affected parts are easily spotted by their sickly yellowish appearence and crustaceous galls (Fig. 24). On drying, the entire plant—healthy as well as diseased—turns black and as a result the herbarium specimens show no symptoms of the disease. Sporangial galls on dehiscence give rise to crusts while the hypnospore galls remain less conspicuous (Fig. 22, 23).

Synchytrium maculans sp. nov.

Prosori sphaerici, pallide fulvi, parietibus tenuibus praediti, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 50 vel plura, sphaerica vel ovata, 24 μ diam., lutea. Hypnosporae sphaericae 82–125 μ (100 μ) diam., fusco-brunneae, contentis luteis, globularibus. Exosporium brunneum, 6 μ crassum. Gallae sporangiales punctiformes, hemisphaericae, 0.2–0.4 mm. diam., post dispersionem cupulatae, multicellulares, rare aggregatae, luteolae. Gallae hypnosporarum multicellulares, 0.2 mm. diam., brunneae. Hypnosporae et prosori singuli vel multi pro galla, singuli pro cellula hospita. Partes infectae haud incrassatae.

Prosori spherical, thin walled, dull brown. Sporangia 50 or more, spherical, or oval, yellow and 24 μ diam. Hypnospores spherical, 82–125 μ (100 μ) diam., dark brown with yellow globular contents. Exospore 6 μ thick. Sporangial galls punctiform, hemispherical, 0.2–0.4 mm. diam., multicellular and light green. Hypnospore galls hemispherical, and centrally depressed, multicellular, 0.2 mm. diam., brown and hard. Hypnospores and prosori one or more in a gall, one in a host cell. Affected parts not distorted.

On leaves, petioles, and stem of *Sida rhombifolia* Linn. September 27, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 25, 26, 27, 28).

Periodically flooded waste lands were excellent locations to find young infected *Sida rhombifolia* plants. The galls are single and generally not coalescent and cause no deformations of the affected parts (Fig. 25). Other malvaceous plants such as *Sida cordifolia*,

Sida sp., and *Hibiscus* sp., which were growing in the neighborhood however remained uninfected. A few typical galls were produced on *Althea rosea* (Hollyhock) when artificially inoculated with zoospores. This species differs from *S. australe* Speg. in the hypnospore measurements and sporangial characters and from *S. hibisci* Gupta & Sinha in the formation of prosori and the thickness of hypnospore wall. Cross inoculation experiments are in progress.

Synchytrium cassiae sp. nov.

Prosori sphaerici, parietibus tenuibus praediti, pallide brunnei, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 20 vel plura pro soro, sphaerica, 15–21 μ diam., dense lutea. Hypnosporae sphaericae, 45–80 μ (66 μ) diam., dense brunneae, ovatae oblongae vel angulatae, contentis luteis globularibus. Exosporium 6 μ crassum contentis cellulae hospitae dense fuscum. Gallae sporangiales, multicellulares, ovatae, cupulatae, post dispersionem aggregatae, 0.4–0.6 mm. diam., et rubicundae. Plures hypnosporae nullas gallas gerentes. Ramuli vel folia infecta conspicue incrassatae et rubicunda.

Prosori spherical, thin walled, light brown. Sporangia 20 or more per sorus, spherical, 15–21 μ diam., and deep yellow. Hypnospores spherical, oval, angular or oblong, 45–80 μ (66 μ) diam., with yellow globular contents. Exospore 6 μ thick, with a thick deposition of denatured host cell contents. Sporangial galls, multicellular, oval, erumpent after dehiscence, aggregate, pinkish and measure 0.4–0.6 mm. diam. Galls may not be produced by the hypnospores but cause dirty brown granular appearence of leaf surface.

On tender aerial parts of *Cassia pumila* Lam. causing much hypertrophy and pinkish coloration. September 3, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 44, 45, 46).

The infected tender shoots become pinkish, hypertrophied and appear conspicuous. These symptoms indicate the development of abundant sporangial stages (Figs. 44, 45, 46). The hypnospores are found mostly on the under surface of the older leaves later in the season. They may not cause any galls but appear brownish and granular to the naked eye. The hypnospores in such galls are much smaller and are found in almost all epidermal cells. When the hypnospores are well spaced typical subspherical, multicellular galls are produced (Fig. 45). The cupulate galls may become piliate due to rapid elongation of the gall cells (Fig. 46).

Synchytrium oldenlandiae sp. nov.

Prosori sphaerici, parietibus tenuibus praediti, brunnei, leves, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 100 vel plura pro soro, pyriformia, 18 x 30 μ magnit., citrina. Hypnosporae sphaericae, 85–150 μ (120 μ) diam., leves, dense brunneae, contentis luteis granularibus. Exosporium 9 μ crassum. Gallae sporangiales ellipticae, cupulatae, 0.2 x 0.4 mm. magnit., aggregatae, crustaceae et brunneae. Gallae hypnosporarum fusco-brunneae, separatae, multicellulares et 0.2 mm. diam. Prosori vel hypnosporae singuli pro galla vel cellula hospita. Partes infectae haud incrassatae.

Prosori spherical, thin walled, brownish. Sporangia 100 or more,

pyriform or one end elongated, 18 x 30 μ and orange colored. Hypnospores spherical, 85–150 μ (120 μ) diam., deep brown with yellow granular contents. Exospore 9 μ thick. Sporangial galls elongate, multicellular, coalescent, brown and 0.2 x 0.4 mm. magnit. Hypnospore galls, punctiform, separate, multicellular, deep brown and 0.2 mm. diam. Prosori and hypnospores one in a gall and one in a host cell. Infected parts are not hypertrophied.

On all aerial parts of *Oldenlandia corymbosa* Lam., September 30, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 29, 30, 31, 32).

Synchytrium biophyti sp. nov.

Prosori sphaerici, pallide citri, parietibus tenuibus praediti, vesiculosi, germinantes sporangiosoros gerentes. Sporangia 40–90 pro soro, sphaerica 14–18 μ diam., lutea. Hypnosporae sphaericae, 100–180 μ (130 μ) diam., dense brunneae, contentis luteis globularibus. Exosporium 8 μ crassum, contentis cellulae hospitae densum. Gallae sporangiales, ovatae, 0.4 x 0.6 mm. magnit., separatae vel rare aggregatae, cupulatae, multicellulares, plerumque hypophyllae, incrassatae. Gallae hypnosporarum hemisphaericae, 0.15–0.3 mm. diam., luteae vel brunneae, nitentes, plerumque hypophyllae, haud incrassatae.

Prosori spherical, pale orange, thin walled. Sporangia 40–60 in number, yellow, spherical and 14–18 μ diam. Hypnospores spherical, 100–180 μ (130 μ) diam., bright brown with yellow globular contents. Sporangial galls oval, 0.4 x 0.6 mm., separate, rarely crowded, multicellular, occur usually on the under surface of the leaflets causing a pink area on the other surface. Hypnospore galls, hemispherical, 0.15–0.3 mm. diam. multicellular, yellow to brown, glistening, separate and occur mostly on the under surface of the leaflets.

On the leaflets of *Biophytum reinwardtii* Edg. & Hook. f. September 30, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 33, 34, 35, 36).

Synchytrium rhynchosiae sp. nov.

Hypnosporae sphaericae, 115–180 μ (150 μ) diam., fusco-brunneae, contentis luteis globularibus. Exosporium 10 μ crassum, contentis cellulae hospitae defunctis densum. Gallae hemispericae, 0.3–0.7 mm. diam. leves, aggregatae, luteae, multicellulares, post dispersionem hypnosporarum cupulatae. Hypnosporae singulae pro galla vel cellula hospita.

Hypnospores dark brown, spherical, 115–180 μ (150 μ) diam., with yellow globular contents. Exospore 10 μ thick, with a thick deposition of denatured host cell contents. Galls hemispherical, multicellular, 0.3–0.7 mm. diam., yellow and much crowded. Hypnospores single in a gall, single and free in a host cell and leave behind cupulate galls on dispersal.

On all aerial parts of *Rhynchosia aurea* DC. September 30, 1951. Banaras, India. Leg. B. T. Lingappa. (Figs. 37, 38, 39).

This species presents an unusual instance of dispersal of hypnospores at maturity. The hypnospores at maturity are pushed upward by the underlying hypertrophied host cells and the hypnospores come off at the slightest friction. Casual rubbing of the palm on the leaf

surface was enough to release hundreds of dark brown resting spores on the palm. This release of hypnospores results in cupulate galls.

Synchytrium viticola sp. nov.

Hypnosporae sphaericae, 165–200 μ (190 μ) diam., contentis luteis globularibus densae. Exosporium 8 μ crassum. Gallae subsphaericae, papillatae, multicellulares, 0.3–1 mm diam., leves, nitentes, pallide luteae, puniceae vel fusco rubrae, culmis petiolisque aggregatae, sed hypophyllae dispersae. Hypnosporae singulae pro galla vel cellula hospita. Ramuli infecti conspicue incrassati.

Hypnospores spherical, 165–200 μ (190 μ) diam., deep brown, with yellow granular contents. Exospore 8 μ thick. Galls subspherical, papillate, raised, hard, pale green to yellow or pink to dark red, 0.3–1 mm. diam., much crowded on stems and petioles and separate on the leaves. Hypnospores one in a gall, one in a host cell. Affected stem and petioles much hypertrophied.

On leaves, stem and petioles of *Vitis trifolia* Linn. September 12, 1950. Banaras, India. Leg. B. T. Lingappa. (Figs. 40, 41).

Repeated examination of the fresh and stained preparations revealed only the resting spores in this species (Fig. 41). The material of *S. parthenocissi* Cook on related hosts is reported to show resting spores measuring 15 (?) to 240 μ diam. and having a thin exospore. It is not clear whether it has any sporangia. Marked hypertrophy is also absent in all hosts of *S. parthenocissi*.

Recently Mishra (1954) described *S. ampelocissi* without a Latin diagnosis. It is reported to produce scabby and warty symptoms on its hosts and resting spores that drop off from the old galls, while *S. viticola* incites the formation of galls which are bold and highly colored and the resting spores never fall off naturally from the old galls as happens typically in the case of *S. rhynchosiae* described above. Except for the these discrepancies, *S. ampelocissi* on further examination may have to be treated as a synonym of *S. viticola*.

A species of *Synchytrium* was collected in 1943 on *Atylosia* sp. and was identified as *S. atylosiae* (Petch) Gäumann (Mhatre and Mundkur 1945). It produces only thin walled sporangiosori and is a member of the subgenus *Woroninella*. The subgenus *Woroninella* is characterized by the absence of resting spores, production of only sporangiosori, and formation of open crateriform and aecidium-like galls (Gäumann 1927). However, the author collected a *Synchytrium* on *Atylosia scarabeoides* plants which showed only hard unopened galls. These galls contained only the resting spores (Fig. 43). No sporangial stages were observed. These characters warrant the conclusion that the species at hand is new and is named after Dr. M. J. Thirumalachar who first collected *S. atylosiae* in India.

Synchytrium thirumalachari sp. nov.

Hypnosporae sphaericae, 132–181 μ (150 μ) diam., singulae, fusco-brunneae, contentis luteis globularibus. Exosporium 7 μ crassum, contentis cellulae hospitae defunctis densum. Hypnosporae singulae vel multae pro galla vel cellula. Gallae subsphaericae, 0.3–0.6 mm.

diam., conspicuae, rubrae, puniceae, vel fusco-brunneae, multicellulares, durae, separatae vel aggregatae, leviter incrassatae.

Hypnospore spherical 132–181 μ (150 μ) diam., solitary, dark brown with yellow globular contents. Exospore 7 μ thick, with a thick deposition of denatured host cell contents. Galls subspherical, 0.3–0.6 mm. diam., multicellular, red, pink or dark brown, hard, separate or aggregate. Hypnospores one or more in a gall but only one in a host cell. Infection causes slight thickening and deformation.

On all aerial parts of *Atylosia scarabeoides* Bth. October 3, 1950. Banaras, India. (Figs. 42, 43).

The types of all 13 species described above will be deposited at the Mycological Herbarium, I. A. R. I. New Delhi; at Commonwealth Mycological Institute, Kew, England; and at the New York Botanical Garden, New York, N. Y.

Most of the information presented in this paper is drawn from the thesis entitled "Contribution to the Knowledge of the Indian Species of the Genus *Synchytrium* De Bary et Woronin," which the author submitted in part-fulfillment of the degree of Master of Science in Agriculture to the Banaras Hindu University in May 1952. The author is very thankful to Dr. Aksheiber Lal for facilities and encouragements and to Dr. J. S. Karling for making valuable corrections in the paper. He is thankful to Mr. D. L. Bohra for drawings of host plants and to Mr. D. Bardis and Dr. Theodor Just for Latin translations of the diagnoses.

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Structure and Dehiscence of the Anther in Exacum pedunculatum L.

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INTRODUCTION

Exacum and the saprophytic *Cotylanthera* are the two genera of the family Gentianaceae showing poricidal dehiscence of anthers. In other genera of this family, dehiscence of the anther occurs in the usual longitudinal manner. In *Exacum* there are two apical pores, one for each half of the anther, whereas in *Cotylanthera* there is a coalescence of the anther halves in the upper part and dehiscence occurs only by a single apical pore.

Two sections are recognized under the genus *Exacum* (Gilg, 1895). Under *Pseudosebaea* Griseb. are grouped the smaller flowered annual herbaceous species whose anthers open at the apex by pores that scarcely extend downward. Examples of this group are *E. pedunculatum*, *E. petiolare* and *E. pumilum*. The second section *Pseudochironia* Griseb. comprises the perennial herbaceous or half shrubby species with large flowers. In this group the anther at first opens apically by two pores which, however, soon continue down its sides as two longitudinal slits. *E. tetragonum* is an example of this section.

MATERIAL AND METHODS

Exacum pedunculatum was available for study in the present instance. Flowers of this plant were collected from a place about forty miles from Mysore city and fixed in formalin-acetic-alcohol. After the customary methods of dehydration and imbedding in paraffin, microtome sections were cut at thicknesses ranging from 6–12μ. Two stain combinations were used, safranin with fast green and Heidenhain's iron-haematoxylin with eosin. Whole mounts of anthers and pollen grains were also used for study.

OBSERVATIONS

The small bluish flowers are tetramerous. The sepals are winged on the back. The globose corolla has a short tube and twisted rotate lobes. The stamens are epipetalous and are inserted on the throat of the corolla. Enantiostyly occurs as in *Cassia*, the style being displaced towards one side or the other of the flower.

The anthers are dithecous, nearly oblong in outline and inserted on a short filament which broadens at its base (Fig. 1). They have blunt tips with two oval pores at the summit (Fig. 2).

The vascular bundle of the filament remains undivided in the connective and ascends to very near the apex of the anther (Figs. 3–6).

The anther wall consists of an epidermis, a hypodermal layer, 2 middle layers and a tapetum. The tapetal cells remain uninucleate and have 1–2 large vacuoles (Fig. 7). At four points on the septa, the tapetal cells are elongated and protrude into the pollen sacs (*it* in fig. 7 shows part of one group of such tapetal cells).

The inner surface of the tapetum shows granular accumulations

which are very prominent at the time when the pollen grains are binucleate (Fig. 8, g). Soon after, the tapetum is absorbed.

The hypodermis gets thickened and serves as the mechanical layer during dehiscence. Reticulate thickenings are laid down in some of its cells (Fig. 8) as well as in some internal cells of the connective. In

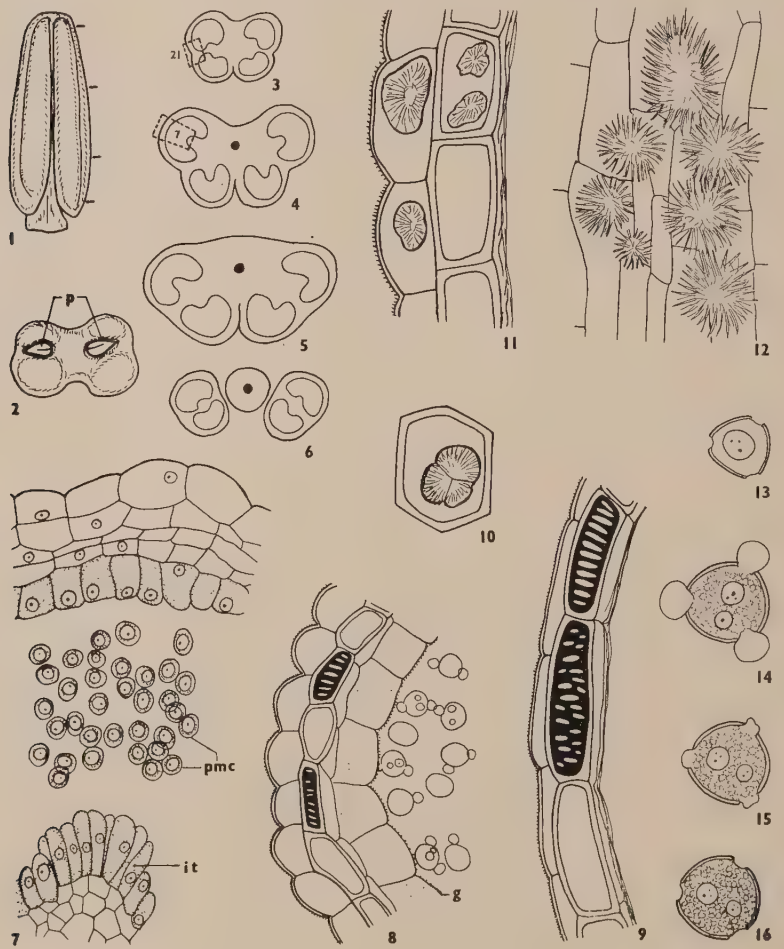


FIG. 1. Young anther. X11. FIG. 2. Apex of dehisced anther as seen from above to show the pores (*p*). X33. FIGS. 3–6. T.S. of young anther from above downwards at levels indicated in FIG. 1. X33. FIG. 7. Part of t.s. young anther magnified from FIG. 4, showing intruding tapetal cells (*it*) of the inner angle of the pollen sac, and pollen mother cells (*pmc*). X300. FIG. 8. Part of t.s. older anther (*g*=granules on the inner surface of tapetum). X333. FIG. 9. Part of t.s. wall of dehisced anther. X618. FIG. 10. A hypodermal cell in tangential view showing a large crystal within. X618. FIG. 11. Part of wall of dehisced anther in t.s. showing crystal in epidermal and hypodermal cells. X618. FIG. 12. Part of v.s. connective to show large brushlike crystals. X333. FIGS. 13–16. Pollen grains in different stages of development. X945.

the mature anther the middle layers are nearly crushed out and the wall consists mainly of the epidermis and the thickened hypodermis (Fig. 9).

Scattered cells of the epidermis and hypodermis show crystals (Figs. 10, 11). In addition there occur large brush-like crystals (Fig. 12) in the vicinity of the vascular bundle of the connective.

In sections of young anthers the sporogenous cells appear in radiating rows in the microsporangia. The pollen mother cells are also similarly disposed till they round off and divide. The entire sporogenous mass is involved in the formation of the pollen mother cells. Divisions of the latter are of the simultaneous type, but occasionally a dark staining transverse band appears between the daughter nuclei at the end of the first division. Some of the divided pollen mother cells may degenerate. The pollen tetrads remain invested for some time by the mother cell wall. A large tube nucleus and a smaller generative cell are formed and starch grains accumulate in the protoplast.

Figures 13–16 show the pollen grains. Figure 13 is of a uninucleate pollen grain. In the early binucleate phase of the pollen, there appears a prominent nodular outgrowth at each of its three germ pores (Fig. 14). After a time, the outgrowths dwindle in size (Fig. 15) and eventually disappear, so that no trace of them is left in the mature pollen grain (Fig. 16). The pollen grains remain binucleate till the time of shedding.

There are two linear but short and transversely placed stomia in whose place the two pores arise later. Each stomium lies at the summit of its anther lobe. The stomia appear as slight depressions overlying the septa of the thecae (Figs. 17, 18). The structure of the stomial regions is shown in figures 19, 20 which represent vertical sections of the anther tip along planes indicated by the arrows in figure 18. In figure 19 the stomium is cut tangentially along its entire length and in figure 20 it has been cut across. The general epidermal cells covering the summit of the anther are radially elongated and have convex, bulging outer walls which later become heavily thickened and cutinized. The epidermal cells of the stomial region, however, remain small and unthickened. The hypodermal cells also remain small in this region and there is an intervening small-celled stomial tissue between the stomial epidermis and the apices of the two pollen sacs in each anther half.

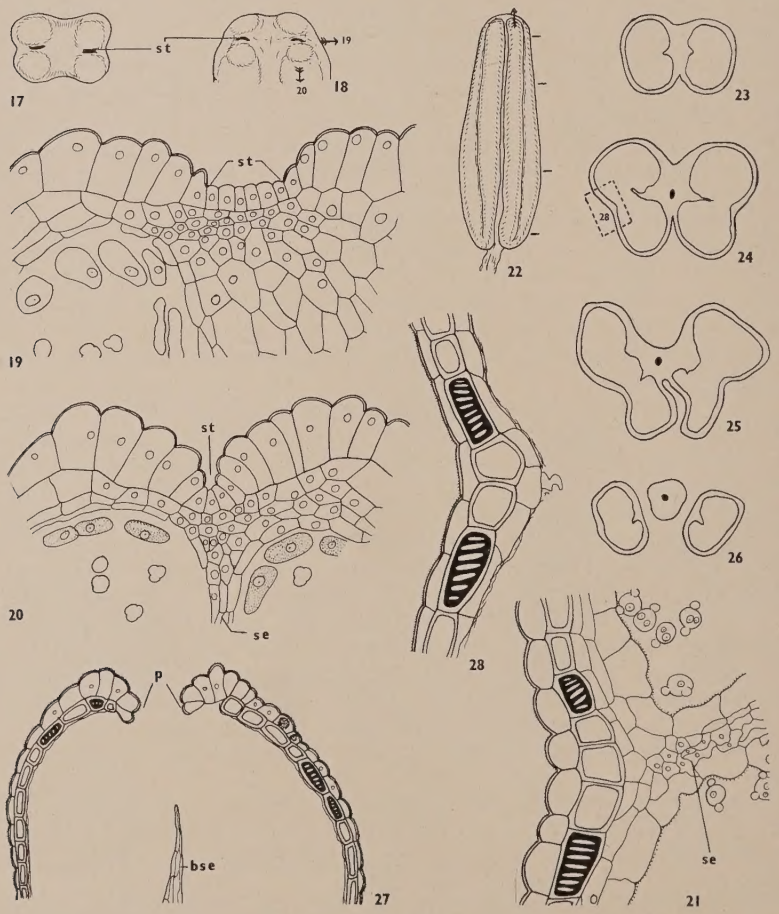
The stomia are short and confined to the apex of the anther. For the rest of its length the anther does not have unthickened areas in its wall even across each septum where the epidermal and hypodermal layers remain uninterrupted (Fig. 21).

Very early in development, part of the septal tissue becomes more or less crushed (*se* in fig. 21). Eventually it is along this area that the septa break down and adjoining pollen sacs fuse. By the disorganization of the stomia and the parts of the septa underlying them, small openings are formed, which broaden into two oval pores by retraction of the broken edges of the anther wall.

Figure 22 is of a dehisced anther and figures 23–26 are serial transections through such an anther at the levels indicated. Figure 27

shows the structural details of the apical part of a dehisced anther. The pore (only one of the two pores is seen in this figure) has formed by the dissolution of the stomia and by the retraction of the broken edges of the wall. The dissolution has also caused the terminal part of the septum to break away from the anther wall.

The lower part of the dehisced anther becomes crumpled (Figs. 25, 26) owing to a general shrinkage of the wall. However, the wall remains unbroken except in the stomial region and the pores remain



FIGS. 17, 18. Top and oblique views respectively of apex of undehisced anther to show the location of the stomia (*st*). X30. FIGS. 19, 20. Parts of v.s. undehisced anther in the two planes indicated in FIG. 18 (*se*=septum between adjacent pollen sacs; *st*=stomium). X300. FIG. 21. Part of t.s. of anther in the region indicated in FIG. 3 (*se*=septum). X300. FIG. 22. Dehisced anther. X10. FIGS. 23–26. T.S. of above at the levels indicated. X30. FIG. 27. V.S. through one of the pores (*bse*=broken septum; *p*=pore). X130. FIG. 28. Part of t.s. dehisced anther in the region marked in FIG. 24. X300.

confined to the apex without showing any tendency to lengthen downwards along the sides of the anther. In figure 28 which shows the structural details of such a region, the general epidermis and the thickened hypodermis are intact and unbroken in a region where an ordinary anther would have split. Thus the failure of a complete dehiscence is due to the lack of stomial areas along the greater length of the anther. The delicate radial walls of many epidermal cells may fold and collapse so that their thick outer walls come to lie over the inner wall and the hypodermis.

CONCLUSIONS

The general course of pollen development in *Exacum pedunculatum* differs in some ways from what has been previously described for other members of the family. In *Gentiana*, *Swertia* and others (Guérin, 1924–26; Wóycicki, 1932) there is extensive sterilization of many of the sporogenous cells which serve for the nutrition of the remaining sporogenous cells. No such sterilization was seen in *E. pedunculatum* although some of the divided pollen mother cells sometimes show degeneration. An interesting feature noted in this species is the presence of ephemeral nodular outgrowths at the germ pores which appear when the pollen is in the early binucleate phase. They dwindle and eventually disappear. No such structures have been reported in other Gentianaceae.

Wóycicki (1932), reports a plasmodial tapetum in anthers of *Gentiana asclepiadea*. In *E. pedunculatum*, however, the tapetum is of the secretory type and its cells remain uninucleate as in other Gentianaceae like *Enicostema littorale* (Srinivasan, 1941).

The poricidal dehiscence of the anther is associated with two very short linear stomia. The latter show no peculiarity except that they are greatly circumscribed in extent and are transversely placed at the summit of the anther. That the pores are not of a highly evolved type, is also evident from the fact that their opening is associated with a hypodermal hygroscopic mechanism as is usual in typical anthers which dehisce longitudinally.

Unlike some other species of the genus (belonging to a different section, *Pseudochironia*), in *E. pedunculatum* (section *Pseudosebaea*) the pores remain confined and do not extend downward as slits. There is no scope for such extension as the thickened hypodermis and the epidermis continue uninterrupted along the middle line of each anther lobe where normally a stomium should have existed. However, in those species where extension of the apical pores does occur, such prolongation to form long slits is probably connected with a weakness in the wall of the anther, due either to rudimentary stomia or because the epidermis and hypodermis are not so constructed as to be effective in restricting the openings to the apex alone. Such species may be held to represent a less highly evolved state of poricidal dehiscence than *E. pedunculatum* and its close allies which show restricted pores.

Cotylanthera, the only other poricidal genus of the family, has gone a step ahead of *Exacum* in the matter of structure and dehiscence of the anther. Here the pollen sacs of the two thecae fuse and open by a

single apical pore and a fibrous layer is not differentiated (Oehler, 1927).

The lower part of the dehisced anther of *E. pedunculatum* shows a crumpling and collapse of the wall, but the spiral twisting of anthers which occurs in species of *Erythraea* and *Exacum* (Rendle, 1925) was not seen in this plant.

SUMMARY

Exacum pedunculatum differs from some other Gentianaceae previously investigated, in certain features of development of the pollen which are described.

The anther dehisces by two oval apical pores which arise in place of two short, linear and transversely placed stomia at the summit of the anther.

The poricidal dehiscence is not of a highly evolved type judging from the fact that the stomia are narrow and linear as in typical longitudinally dehiscing angiospermic anthers. Further, dehiscence is associated with a hypodermal hygroscopic mechanism. The only modification that has occurred therefore is a shortening of the stomia and their restricted development only at the tip of the anther.

The narrow initial openings formed in place of the linear stomia gape widely and assume the form of two oval pores. The pores remain circumscribed and do not extend downwards as slits like those of certain other species of *Exacum*.

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